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2. (To a book)—Doe, J. A., *The Dental Practice* (Chicago, Smith: 1930), p. 114.

See McBride, *J. D. Res.*, **16**: 537, 1937.

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THE EDITOR'S VIEWPOINT

Orban, Ritchey and Zander (p. 299) report on an experimental study of pulp changes in the decompression chamber. Numerous earlier studies were of the observational type. The importance of anoxia in producing hyperemia and capillary hemorrhage has been demonstrated by the present experiments. The footnote on page 307 suggests an unfortunate decision, made without justfication. We trust that this will not be continued in military or other groups.

Dr. Bass (p. 401) has been able to demonstrate, in the gross, a line indicating the place of attachment of the outer border of the epithelial attachment. As the author states, this has probably been observed by others but the present report is the first complete published description. We don't like "zdeac" as a term but can see its value in text. Periodontists should find this paper of special interest. We call your attention to the fact that Dr. Bass is a physician on the faculty of a medical school.

From Zoller Clinic comes a study of fluoride feeding (p. 311) by Greenwood, Blayney, Skinsnes and Hodges. Fluorides, in the form of NaF, purified bone meal and defluorinated phosphate significantly effected the serum Ca and P, Hb, blood coagulation time and growth but only those puppies fed the fluorine as NaF developed dental fluorosis. The teeth of those animals fed the bone meal and defluorinated phosphate were "superior". The why of the last finding is worthy of long discussion.

Irving and Nienaber contribute a study of fluorides (p. 327) from South Africa. Using rats as subjects, they injected NaF and found no effect on the blood Ca and P of normal rats, but significant changes in animals on high or low Ca:P ratio diets. CaF_2 appeared to be deposited in the dentin.

Cadmium was used by Leicester (p. 337) in a caries study of rats. Small quantities of cadmium fed in drinking water were not taken up by the calcifying molars during their period of calcification but when fed in water over 123 days from birth cadmium appears in the molars. The cadmium seemed to increase the caries rate but not the number of cavities.

Keyes presents a rather extensive report on the character and distribution of carious lesions in the hamster (p. 341). He concludes that, fundamentally, hamster and human caries are very similiar but that comparisons must be made with reservations. This paper is from a thesis submitted for the Master of Science degree.

Bevelander and Johnson submit a report in dentinogenesis (p. 381). In view of the many philosophical discussions of the role of the odontoblast, this histochemical study is refreshing.

Staz, another of our South African contributors, discusses the bands of Shreger (p. 373). In light of earlier findings of Beust, Kitchin and Kronfeld, among others, and Diamond and Weinmann, later, we are inclined to consider the two phase development of enamel as the "accepted" view rather than as a modification of the accepted view. The tooth Staz has studied is interesting and the optical phenomenon intriguing.

Gurney and Rapp have developed a technic for observing minute surface changes in enamel (p. 367). The present paper was received a week before a similar technic was described by Scott and Wyckoff (Pub. Health Rep., **61:** 697, 1946). While the technics are similiar each has peculiar advantages.

The paper by Jansen (p. 355) has an interesting background. It was submitted for the author, who is on Java, by Dr. K. C. Winkler of the Hygienisch Laboratorium der Rijksuniversiteit in Utrecht. It was prepared in Holland during the occupation by Germany and was published in the Dutch Language in "Het Nederlandse Tijdsdrift voor Tandheelkunde." Since that period-

ical would be seen by few, if any, of our readers and since so few can read the Dutch language, we have published the paper here. Our own past efforts to make "serial" ground sections whetted our interest in Dr. Jansen's technic. Application of this method might have given the answer to many questions raised in discussion at the 25th General Meeting of the Association.

Bodecker and Lefkowitz (p. 387) present a resumé of their veiws with some additional points on the vitality of dentin and enamel. We are rather inclined to agree with Churchill (Meyer's Normal Histology and Histogenesis of the Teeth, Phila., Lippincott: 1935) when he stated that much of the vitality discussion hinged on a definition of "vitality". As the authors mention the implications are important in operative dentistry and in caries control. We hope that the addendum helps clarify differences of opinion.

The New York group report their proceedings on p. 417.

H. B. G. R.

EXPERIMENTAL STUDY OF PULP CHANGES PRODUCED IN THE DECOMPRESSION CHAMBER[1]

BALINT ORBAN, M.D., D.D.S.,[2] BERYL RITCHEY, D.D.S.[3] AND H. A. ZANDER, D.D.S.[4]

From the Department of Research, School of Dentistry, Loyola University, Chicago College of Dental Surgery, Chicago, Ill.

INTRODUCTION

Following a study of microscopic specimens of human teeth extracted because of pain in simulated flight[5] it seemed advisable to make some experimental studies. Several questions had developed in connection with pulp reaction to cavity preparation and to commonly used filling materials; most of the clinical reports on toothache at altitude stressed the fact that the pain occurred most frequently in teeth recently filled. Animal experimentation was chosen as the method of investigation because of the possibilities it offered for uniformity and repetition. The investigation was planned in an attempt to demonstrate whether decompression in itself would produce tissue changes in healthy pulps, or, whether certain pre-existing pulp changes, possibly due to operative procedures, were a prerequisite for altitude disturbances.

This paper deals with 2 series of experiments. The first study was carried out in April 1945. The specimens then obtained did not yield conclusive results but they directed attention to certain features which prompted a second experiment carried out in November 1945. The experiments are described, discussed, and summarized separately but the conclusions are drawn from an evaluation of both experimental series.

FIRST EXPERIMENT

Material and Method of Investigation

The material studied consisted of the 6 upper and 6 lower incisors of 7 young adult dogs. Under nembutal anesthesia, the dogs received 2 fillings each of silver amalgam, silicate cement, zinc-oxide and eugenol, oxyphosphate cement, and aquadont[6]. In each dog 1 tooth was left without a cavity and in 1 tooth in each dog a cavity was prepared and treated with croton oil to insure a definite

[1] The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the School of Dentistry, Loyola University, Chicago, Illinois. Received for publication April 3, 1946.

[2] School of Dentistry, Loyola University, Chicago, Ill.

[3] Colorado Springs, Colorado.

[4] Tuft's College, Dental School, Boston, Mass.

[5] Orban, B. and Ritchey, B., Toothache under Conditions Simulating High Altitude Flight, *J. A. D. A.* **32:** 145; 1945.

[6] Zinc oxide—100 gm. zinc sulphate—100 gm. and starch—50 gm.; to be mixed with water, an entirely non-irritating filling material.

irritation[7]. Corresponding teeth were used for the same filling material and for the controls. All cavity preparation was done under a stream of water; the enamel was cut through with a knife-edged diamond stone and the dentin cut with a sharp inverted cone bur.

Twelve days after the fillings were placed the 2 control dogs were sacrificed at ground level; 2 dogs were taken to 38,000 feet in the decompression chamber for a period of 30 minutes and were sacrificed 30 minutes after they were returned to ground level; and 2 dogs were taken to 38,000 feet for a period of 30 minutes and sacrificed at this altitude in the decompression chamber. One dog succumbed in the decompression chamber at an altitude of about 32,000 feet during the ascent. All dogs were under the action of nembutal during the decompression. Oxygen was supplied by means of a head box. The animals were sacrificed by the injection of sodium cyanide into the heart.

Immediately following the sacrifice of each dog the teeth were dissected out and ground, under water, to thin discs and fixed in freshly mixed Zenker-formalin (Maximow). The specimens obtained in the decompression chamber at 38,000 feet were placed in the fixing solution and sealed in a desiccating jar so that the fixation took place under decompression[8].

Findings

1. Control dogs sacrificed at ground level. No significant difference in these pulps could be seen irrespective of the filling material which had been used. The only inflammatory reaction was found in the tooth which had been treated with croton oil. In 1 tooth filled with aquadont (*fig. 1*) there was a slight hyperemia in the pulp just below the cavity.

The specimen shown in *fig. 2* is from a tooth filled with silver amalgam. The odontoblasts are undisturbed below the floor of the cavity. The pulp shows no inflammatory reaction. The other filling materials caused no appreciable irritation; the findings in both of these control animals were practically identical.

2. Dogs sacrificed at ground level 30 minutes after decompression. In respect to reaction to the filling materials these dogs resembled the 2 control animals. A few minor changes could be seen in the odontoblasts below the cavities in some instances but they could not be attributed to any one filling material because they were observed in a number of different specimens. Some minor inflammatory reactions were occasionally observed. The coronal part of the pulp was often entirely normal, neither hyperemic nor edematous. In the middle third of the root canal (*fig. 3*) a hyperemia was often observed; the vessels were widened and filled with blood or plasma. Only rarely were minor hemorrhages observed. The apical portions of these pulps were usually entirely free from hyperemia.

[7] Robinson, H. B. G. and Boling, L. R., Anachoretic Effect in Pulpitis. I. *J. A. D. A.* **28:** 268; 1941.

[8] The decompression experiments were carried out in the School of Public Health of Harvard University under the supervision of Dr. Cecil K. Drinker. It is the pleasant duty of the authors to express their appreciation and thanks to Dr. Drinker for permission to use the decompression chamber and for his suggestions and interest.

3. Dogs sacrificed at 38,000 feet and specimens fixed at this altitude. All specimens, regardless of the filling material used, presented extensive hyperemia; the vessels were greatly distended and filled to capacity with blood (*fig. 4*). Two teeth in the upper jaw showed bubble formation (*fig. 5*); there is little hemorrhage around the distended vessels but the "bubble" is surrounded by red blood corpuscles. In some specimens small capillary hemorrhages could be seen. A hyperemia extends into the minute capillaries between the odontoblasts; it was not more extensive below the floor of the cavity than in other

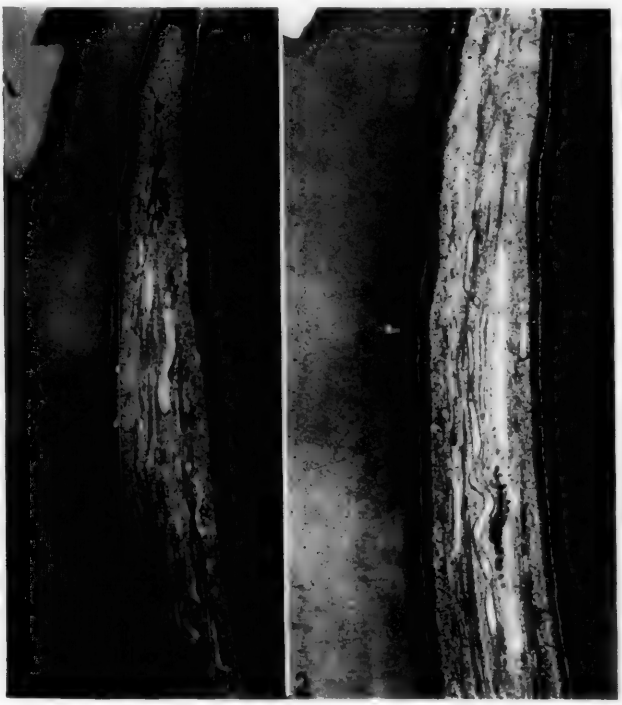

FIG. 1 FIG. 2

Fig. 1. AE $\frac{5}{2}$ Aquadont. Ground level control. Slight hyperemia below the cavity (a)
Fig. 2. AE $\frac{4}{1}$ Amalgam. Ground level control. No patholgic changes in the pulp

portions of the pulp. The untreated control teeth showed a similar reaction (*fig. 6*).

4. Dog that died at 32,000 feet. The specimens obtained from this animal were most unusual; a severe hyperemia with extensive hemorrhages characterized the microscopic findings. *Fig. 7* shows a tooth which had been filled with silver amalgam; the vessels are wide and filled to capacity with blood. Capillary hemorrhages occurred all along the odontoblastic layer. There is no indication that either the cavity preparation or the filling material had anything to do with the hemorrhages. In another tooth (*figs. 8* and *9*) filled with aquadont, hemorrhages

are seen throughout in the pulp; there is an almost continuous hemorrhage between the odontoblasts. In several teeth from this animal the hemorrhages were especially pronounced below the floor of the cavity (*fig. 10*) though they were not limited to that area.

Discussion

The pulp changes apparently occurred without respect to the type of filling material used; croton oil, however, produced a definite inflammatory response.

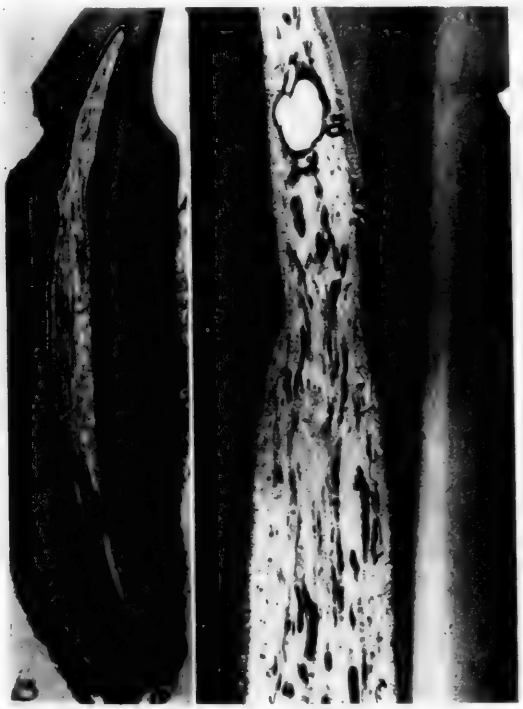

FIG. 3 FIG. 4

Fig. 3. AA 4 |1 Amalgam. 38,000 feet and returned to ground level. Hyperemia in middle third of root canal (a). No other pathologic changes.

Fig. 4. AF 2 2| Aquadont. 38,000 feet; fixed at altitude. Extensive hyperemia in entire pulp; "Bubble" in pulp horn (a).

Comparison of these data with human pulp reactions under decompression is difficult. All fillings in this experiment were placed in freshly cut cavities in sound teeth, whereas, in the human material previously studied[5], all specimens had presented carious lesions prior to restoration; there were no records of pulp disturbances which may have existed in the human material prior to restoration. Comparison to human response under similar conditions is also difficult because the pain symptom is missing in the experimental animals. All conclusions are necessarily based on histopathologic findings. Since no pulp changes of

consequence could be attributed to the operative procedures all changes observed could only be the result of the decomprssion experiment.

Only a slight hyperemia was observed in the pulps of those animals which were returned to ground level following decompression and sacrificed thirty minutes later; without other specimens for comparison it could almost be concluded that nothing had happened to these pulps as a result of the decompression, or, that whatever changes might have occurred had subsided after the return to ground level. *Figs. 4, 5,* and *6* show specimens taken at altitude; it is very possible that a hyperemia and edema of this degree could almost entirely disappear in the time elapsing between decompression and sacrifice at ground level.

FIG. 5 FIG. 6 FIG. 7

Fig. 5. High magnification of "bubble" form *fig. 4.* Hemorrhage around bubble (a).

Fig. 6. AF 9 $\overline{1}$| Control tooth (no cavity). Fixed at 38,000 feet. Extensive hyperemia in entire pulp.

Fig. 7. AC 3 _1| Amalgam. Animal expired at 32,000 feet due to acute anoxia. Extensive hyperemia and hemorrhages especially in the odontoblastic layer (a).

There is another feature in the experiment which makes a clear cut conclusion difficult. The animal that died on its way up, at 32,000 feet, shows findings similar in nature to those of the 38,000 feet specimens but the changes are considerably exaggerated; the hyperemia is more severe and the hemorrhages are enormous. The death of this animal was thought to be due to an acute anoxia resulting from a poor adjustment of the oxygen apparatus. An apparent lack of oxygen was also noted in the animal from which *figs. 4, 5,* and *6* were taken; artificial respiration was applied, the animal resumed normal breathing, com-

FIG. 8

FIG. 9

Fig. 8. AC 5 |2 Aquadont. Animal expired at 32,000 feet. Almost continuous hemorrhages between odontoblasts (a and b).

Fig. 9. High magnification from area (b) from *fig. 8*. Hemorrhage between the odontoblasts (a); Distended blood vessels (b).

Fig. 10. AC 11 |2 Cement. Animal expired at 32,000 feet. Hemorrhages especially below the floor of the cavity (a).

pleted the ascent to 38,000 feet, and remained there for 30 minutes before it was sacrificed. The other dog taken to simulated high altitude had no noticeable respiratory difficulties; the pulp specimens from this dog showed a hyperemia and a few hemorrhages but not as extensive as those observed in the pulps of either of the other altitude dogs.

The mid-root hyperemia noted in many of the specimens can be explained by the peculiar arrangement of the vessels of the dog pulp. The blood vessels enter the pulp canal through several small openings (8–14) into the root apex. The arteries ascend into the pulp as straight trunks but begin to divide in about the middle third of the root and become wider. Reaching into the coronal portion of the pulp chamber, the vessels further divide into fine branches and capillaries. The hyperemia was often seen to be located in the mid-third where both arteries and veins have their main branching and where the vessels appeared thin-walled and widest. Toward the apex the veins become thicker-walled straight and narrower, to permit their passage through the apical foramina. A thorough study of blood vessels in dogs and cats has been published by Boling[9] of the First Experiment.

Summary of the First Experiment

1. Experimental fillings, using the common restorative materials, were placed in the incisor teeth of dogs and the pulps of these teeth examined microscopically.

2. Two dogs were used as ground level controls; 2 were sacrificed on the ground following a 30 minute decompression; 2 dogs were sacrificed at altitude; and 1 dog died, presumably of acute anoxia, during ascent. All altitude specimens were sealed in an air tight jar to insure tissue fixation under decompression.

3. There was no appreciable difference in the response of the pulp to the variety of filling materials used.

4. Croton oil produced a definite inflammatory response when applied to dentin exposed in freshly prepared cavities.

5. A pronounced hyperemia and occasional small hemorrhages occurred in the pulps of the animals sacrificed at 38,000 feet in the decompression chamber.

6. A very severe hyperemia and multiple focal hemorrhages occurred in the pulps of the animal which succumbed during ascent. The death of the animal was thought to have been caused by an acute anoxia.

7. No definite conclusion could be reached as to whether the hyperemia and hemorrhages should be attributed to decompression or to anoxia.

SECOND EXPERIMENT

In an attempt to distinguish between the histopathologic effects of decompression as compared with those of acute anoxia the following experiment was carried out[10].

[9] Boling, L. R., *Anat. Record* **82**: 25; 1942.

[10] This experiment was carried out in November 1945 at the School of Aviation Medicine, Randolph Field, Texas. Acknowledgement and thanks are due to Captain David F. Mitchell, D. C. for this assistance in carrying out the project.

Material and Method of Investigation

Six dogs each received an aquadont filling and a croton oil treated cavity (also filled with aquadont) in the incisor teeth of each quadrant of the mouth; the remaining incisor of each quadrant was reserved as a control tooth. Dog No. 1 was provided with a suitable oxygen mask and sacrificed after 15 minutes at 38,000 feet; the specimens were fixed at altitude as in the first experiment. Dogs Nos. 2 and 3 were sacrificed at ground level by a 30 minute asphyxiation with nitrogen administered by tracheal canule.

FIG. 11 FIG. 12

Fig. 11. D 3, Specimen 31. Croton oil. Animal asphyxiated at ground level. Extensive hemorrhages between odontoblasts (a) and in other parts of pulp (b).

Fig. 12. D 3 Specimen 32. Control tooth, no cavity. Extensive hemorrhages (a)

To eliminate variations in the response of different animals the following procedures were carried out on dogs Nos. 4 and 5: In animal No. 4 the lower teeth were removed by mandibular resection under ground level conditions; hemorrhage was controlled by the use of fibrin foam and sutures. While still under the nembutal anesthesia this dog was fitted with the oxygen mask, taken to 38,000 feet and sacrificed there after 15 minutes. The maxillary teeth were then removed and sealed in the fixing solution. In dog No. 5 the lower teeth were removed by mandibular resection under normal ground level conditions

and the upper teeth were removed after a 30 minute asphyxiation with nitrogen also at ground level. Dog No. 6 was used as a control. Microscope preparations were made from all specimens[11].

Findings

All teeth obtained from the asphyxiated dogs showed hyperemia and hemorrhages; both conditions were more or less independent of cavity preparation or the use of croton oil, even the control teeth showed similar findings (*Figs. 11* and *12*). There is some indication that if an irritation is present the hyperemia and the hemorrhage is more extensive.

All pulps obtained from the animals sacrificed at 38,000 feet show some hyperemia. This was especially clear in animal No. 4 where a comparison was possible of teeth in the same animal on ground level and altitude. Hyperemia and hemorrhages in the asphyxiated dogs were not dependent on altitude.

The control animal showed no noteworthy findings.

Discussion

The second experiment involved fewer variables, was better controlled, and the results are more conclusive. It seems apparent that decompression, as carried out in these experiments, produced hyperemia and occasional hemorrhages in the pulp.

There can be no reasonable doubt that the pulp changes observed in the dogs asphyxiated on ground level resulted from an acute anoxia.

It seems equally apparent that the microscopic findings in the pulp, following a fatal anoxia, are the same regardless of whether the anoxia occurred at ground level or at altitude.

The histopathologic changes observed in the teeth of the dogs that were sacrificed during decompression differ from those of dogs asphyxiated at ground level only in degree. The severity of the hyperemia and the number and the size of the hemorrhages appears to parallel the observed respiratory difficulties. In the animals that required artificial respiration at altitude the hyperemia is very pronounced and the hemorrhages rather numerous; the animal believed to have died at 32,000 feet from anoxia presented an extensive hyperemia and multiple hemorrhages.

In addition to an anoxia from failure in the proper adjustment of the oxygen apparatus there is another factor which may have contributed to an anoxia in all of the altitude animals. The decompression tests were carried out under nembutal anesthesia and the respiratory depression, incident to the use of this drug, might have played a part in the development of an anoxia. Since a low barometric pressure, in the cases of apparently well-oxygenated animals, pro-

[11] As originally planned, the experiment called for a complete autopsy of the animals for the purpose of correlating pulpal changes with those of other organs and tissues. Unfortunately permission to examine any but the oral tissues was refused by the medical officer in charge and hence the investigation was limited to the oral cavity.

duced only a mild hyperemia and since the degree of hyperemia and of the hemorrhage was parallel to the respiratory difficulty one is led to suspect that all the findings in these experiments might have been caused by anoxia and not by decompression as such.

It is an established physiologic principle that anoxia results in hyperemia, apparently in an effort to compensate for the lack of oxygen and it is well known that capillary permeability and fragility are freatly increased in anoxia. The histopathologic findings were exaggerated in specimens with a pre-existing inflammation, a finding which could be expected in the light of the known increase of capillary permeability in inflammation.

This material presents cases of an acute nature, i.e. a single ascent at 4,000 feet per minute. One could imagine that less severe circulatory changes, for instance, edema without cellular infiltration or hemorrhage might result from a slower ascent or in animals not under the effect of a respiratory depressant: such findings would be comparable to those observed in specimens of human pulps obtained immediately following decompression.[12]

In view of the fact that bubble formation was not a significant and constant feature of the microscopic findings whereas circulatory changes were a consistent finding, and, in view of the fact that the intensity of circulatory changes paralleled the observed respiratory difficulties one is easily led to the interpretation that a local or general anoxia, rather than nitrogen liberation, might be the dominant factor in the pathogenesis of many aspects of decompression sickness.

Summary of the Second Experiment

1. The pulps of the incisor teeth of 6 dogs were studied microscopically to compare the effects of decompression and of an acute fatal anoxia. Some teeth were treated with croton oil, applied to prepared cavities, to insure an inflammatory response in the pulp; thus a comparison of the response of normal and inflamed tissue was possible. By mandibular resection it was possible to obtain control specimens from the same animal both in experiments with decompression and asphyxiation.

2. As compared to control specimens the pulps obtained at altitude showed a hyperemia and occasional small hemorrhages; irritation by croton oil increased the severity of the hyperemia and the hemorrhages. The existence of a mild anoxia in the altitude animals can not be definitely ruled out.

3. All teeth from dogs asphyxiated at ground level showed severe hyperemia and extensive hemorrhages.

CONCLUSIONS

1. There was no significant difference in the response of the pulps of dogs to the commonly used filling materials; in no case was there more than a mild local tissue reaction. Croton oil applied to cavities produced an acute inflammation.

[12] Ritchey, B. and Orban, B., Toothache at Altitude, *J. Endodontia* **1**: 13, 1946.

2. Decompression to simulate an altitude of 38,000 feet produced a hyperemia and occasional henorrhages; these changes apparently had largely subsided 30 minutes after decompression. The findings were the same in the control teeth and in those subjected to common dental operative procedures; the reactions were increased in intensity in the specimens treated with croton oil.

3. Anoxia, leading to asphyxiation, produced severe hyperemia and extensive capillary hemorrhages; the effects of the anoxia were the same whether it took place at ground level or at altitude.

COMPARATIVE STUDIES OF THE FEEDING OF FLUORIDES AS THEY OCCUR IN PURIFIED BONE MEAL POWDER, DEFLUORINATED PHOSPHATE AND SODIUM FLUORIDE, IN DOGS[1]

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Recent interest in fluorine compounds can be traced primarily to the important observations which follow: (a) The presence of 1 part per million of fluorine in drinking water produces a very mild type of dental fluorosis in about 10% of the population if the fluorine is consumed during the period of the calcification of the teeth. On the other hand, the incidence of dental caries is less in communities with water supplies containing certain levels of fluorine. One part per million of fluorine as sodium fluoride will be added to water supplies in at least 3 different American cities to determine if there is a reduction in the incidence of dental caries. (b) Excessive fluorine has been found in phosphatic mineral supplements which are fed to farm animals and added to the soil as a source of calcium and phosphorus. (c) Specially prepared bone flour containing fluorine has been recommended and used to a limited extent as a source of calcium and phosphorus in the diet of children and pregnant women. (d) In the United States, fluorine compounds (cryolite and barium fluosilicate) are used as insecticidal sprays for fruits and vegetables. (e) Fluorides are employed in the studies of the intermediate metabolism of carbohydrates in animal tissues and micro-organisms.

General reviews on this subject have been prepared by De Eds (3), McClure (18), Roholm (22, 23), Peirce (21), and Greenwood (13). Publications (7, 8, 9) dealing more specifically with the relation of fluorine to dental health summarize the work of several investigators in this field.

Barrentine, Maynard and Loosli (1), Fraser, Hoppe, Sullivan and Smith (10), and Ellis, Cabell, Elmslie, Fraps, Phillips and Williams (5) have recently made nutritive evaluations of defluorinated phosphate as the primary source of calcium and phosphorus in the diet of rats. Bird, Mattingly, Titus, Hammond, Kellogg, Clarke, Weakley and Van Landingham (2) have made a similar study of defluorinated phosphate supplements in the diet of chicks. Shrewsbury and Vestal (24) have reported a comparative study of different phosphate supplements in the

[1] This investigation was supported by a grant to The University of Chicago by The Armour Laboratories, Chicago, Illinois. It is part of an extensive study of the effects of fluorides in animals and man. Received for publication July 8, 1946.

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rations of hogs. In general, these studies indicate that when the phosphorus in the supplements occurred primarily in the ortho form, availability to animals was high, while in the meta or pyro form the availability ranged from reasonably good to poor. Steamed bone meal was superior to defluorinated phosphate in rations of growing pigs (24).

Harootian (14) fed 3 five grain capsules of bone flour daily for 9 months to 9 psychotic patients living in a hospital. He reported a striking diminution in the carious process in the patients. This preliminary investigation, although lacking proper controls, indicated that additional studies should be conducted to determine if the consumption of bone flour would reduce the incidence of dental caries in man and animals.

The purpose of this paper is to report the effects in dogs of feeding fluorides as found in purified bone meal powder, defluorinated phosphate, and sodium fluoride on serum calcium and phosphorus, hemoglobin, blood coagulation time, teeth, bones, and on growth of the animals.

EXPERIMENTAL

Materials and Methods. Mothers of the puppies, with one exception, were obtained from the dog pound. No record was available regarding their ages or origin. They were mongrels and the average weight was about 10 kilograms (range 4 to 20). The animals were kept in cages with wooden or concrete floors. Wood shavings were generally used for bedding. During good weather the dogs were allowed out of doors in 2 large pens with concrete floors.

The dogs received a basal ration consisting of a commercially prepared dehydrated dog food (Pard), fresh beef lungs (from U. S. inspected plant), evaporated milk (Carnation), cod liver oil, and Chicago city water ad libitum. The various supplements were mixed in the basal ration and fed each day. The adult dogs were fed once a day and the young puppies twice. The control dogs and those receiving the fluorine supplements were fed the same basal ration.

The different supplements were fed at a level to provide 5 mgm. of fluorine per kilogram of body weight daily. The fluorine content, in parts per million, of the basal ration and supplements was as follows: Dehydrated feed (Pard) 17.6, fresh beef lungs ca. 0.1, bone meal powder 633.0, defluorinated phosphate (Defluorophos) 588.0, and U.S.P. calcium phosphate 11.0. The basal ration for dogs after 60 days of age contributed about 0.2 mgm. of fluorine per kilogram of body weight daily. The animals fed the supplement containing fluorine plus the basal ration consumed about 5.2 mgm. of fluorine per kilogram of body weight daily. This level was fed to determine if large amounts of the different materials containing fluorine would produce toxic symptoms.

Large quantities of bone meal powder and other calcium-phosphorus combinations have a tendency to produce constipation. This tendency was counteracted by feeding the dogs fresh ground beef lungs from a plant inspected by the United States Government.

An attempt was made to supply the various nutrients at levels recommended by Michaud and Elvehjem (19). Some nutrients, however, were fed at much

higher levels than those suggested by these authors. In order to add 5 mgm. of fluorine per kilogram of body weight daily as bone flour or defluorinated phosphate (Defluorophos) it was necessary to give the animals many times their daily requirement of calcium and phosphorus. A low fluorine containing U.S.P. tertiary calcium phosphate was added to the ration of one group of control dogs at levels which would provide calcium and phosphorus at approximately the concentration obtained by dogs fed bone meal powder or Defluorophos. It was necessary to evaluate the influence of the high concentrations of calcium and phosphorus in the dogs.

The purified bone meal powder was a high grade pharmaceutical product made from beef bones by The Armour Laboratories, Chicago. The defluorinated phosphate (Defluorophos) was manufactured and furnished by the International Minerals and Chemical Corporation, Chicago. Most of the phosphorus in this product was in the ortho form. The percentages of calcium in the bone meal powder and in the defluorinated phosphate were 34.7 and 36.6, and the phosphorus values were 15.7 and 13.7 respectively.

TABLE I

Summary of dogs used in experiments

GROUP	NO. OF LITTERS	NO. AUTOPSIED	NO. ALIVE	TOTAL
Control	4	11	10	21
Bone meal powder	7	15	21	36
Defluorophos	6	11	11	22
Sodium fluoride	4	16	4	20
Totals	21	53	46	99

Samples of blood were obtained from the external saphenous vein of the mothers and puppies. Blood studies were conducted on the puppies during the period of calcification and eruption of the teeth. The coagulation time of the blood was obtained by the capillary tube method. Hemoglobin determinations were made by the Tallquist and spectrophotometric method of Drabkin and Austin (4). Calcium and phosphorus determinations were made on the serum by the methods as described by Koch and Hanke (16).

Fluorine determinations were made essentially by the method of Willard and Winter (26) using the modifications of the Association of Official Agricultural Chemists (6). Magnesium acetate was employed as the fluorine fixative. Potassium fluosilicate was used as the primary fluorine standard. It was prepared and its purity tested in the laboratories of the United States Food and Drug Administration, Washington, D. C.

Roentgenograms were made under pentobarbital anesthesia of the femurs and tibias of representative dogs at approximately 3 months of age and about every 3 months thereafter. Roentgenograms were also made of the jaws and teeth of representative dogs which were sacrificed for histological study.

TABLE II

Blood studies on dogs

DOG NO.	SEX	LITTER NO.	5/5/45					6/2/45					6/23/45					8/4/45					9/15/45				
			Age	Hb	Ca	P	Coag. time	Age	Hb	Ca	P	Coag. time	Age	Hb	Ca	P	Coag. time	Age	Hb	Ca	P	Coag. time	Age	Hb	Ca	P	Coag. time
Controls																											
			days	*gm. %*	*mgm. %*	*mgm. %*	*sec.*	*days*	*gm. %*	*mgm. %*	*mgm. %*	*sec.*	*days*	*gm. %*	*mgm. %*	*mgm. %*	*sec.*	*days*	*gm. %*	*mgm. %*	*mgm. %*	*sec.*	*days*	*gm. %*	*mgm. %*	*mgm. %*	*sec.*
42	M	11	174	15.6	11.8	5.9	60	202	Used for study																		
43	M	10	196	15.6	11.5	5.8	60	224	14.1	11.5	5.8	60	245	15.6	11.8	5.5	60	287	15.6	11.5	5.2	65	329	15.6	10.8	5.2	65
44	M	10	196	15.6	11.5	5.8	50	224	15.6	11.5	5.8	65	245	15.6	11.4	5.2	60	287	15.6	11.4	5.4	60	329	15.6	10.8	5.0	70
Controls with added U.S.P. tricalcium phosphate																											
45	F	11	174	15.6	12.0	6.2	65	202	15.6	12.3	5.8	65	223	15.6	11.7	5.2	65	265	15.6	11.8	5.4	65	307	15.6	11.4	5.4	60
46	F	11	174	15.6	12.0	5.8	60	202	14.1	12.3	6.0	60	223	15.6	11.5	4.8	60	265	15.6	11.6	5.4	60	307	14.1	11.6	5.4	65
Bone flour group																											
47	F	3	197	15.6	12.3	6.0	60	225	15.6	12.5	5.8	65	246	15.6	12.5	5.8	60	288	15.6	12.0	5.6	65	330	14.1	11.8	5.4	60
48	M	13	52	12.5	12.0	6.3	40	80	14.1	12.5	6.3	60	101	14.1	11.8	6.0	50	143	15.6	11.8	5.8	65	185	15.6	11.6	5.6	60
49	F	13	52	12.5	12.5	6.2	40	80	12.5	12.0	5.8	60	101	15.6	12.0	6.0	50	143	14.1	11.7	5.6	60	185	15.6	11.5	5.5	60
50	F	13	52	14.1	12.8	6.3	50	80	12.5	10.8	5.8	60	101	15.6	11.6	5.9	50	143	12.5	11.8	5.7	60	185	14.1	11.6	5.5	65
51	F	13	52	12.5	12.5	6.2	40	80	12.5	12.0	6.0	60	101	12.5	12.0	5.8	45	143	14.1	11.4	5.6	60	185	14.1	11.2	5.4	65
52	F	13	52	12.5	12.0	5.8	40	80	12.5	12.0	6.2	55	101	15.6	11.5	5.6	50	143	14.1	11.8	5.7	60	185	14.1	11.6	5.5	65
53	M	13	52	12.5	12.3	6.0	40	80	12.5	11.8	5.9	60	101	15.6	11.7	5.9	50	143	14.1	11.5	5.8	60	185	15.6	11.4	5.6	65
54	M	2a	33	12.5			50	61	12.5	11.5	6.0	50	82	15.6	12.0	6.4	60	124	14.1	11.7	5.7	60	166	15.6	11.5	5.5	65
55	F	2a	33	12.5			60	61	12.5	11.5	5.9	60	82	12.5	12.5	6.4	50	124	Used for study								
56	F	2a	33	12.5			50	61	12.5	12.0	6.0	60	82	12.5	11.8	6.0	50	124	12.5	11.8	5.8	60	166	14.1	11.6	5.6	65
57	M	2a	33	12.5			60	61	12.5	11.5	5.8	50	82	12.5	12.0	6.0	50	124	14.1	11.6	5.7	65	166	14.1	11.4	5.5	65
58	F	3a	32	12.5			50	60	12.5	11.5	5.8	60	81	12.5	11.7	5.9	50	123	Used for study								
59	F	3a	32	12.5			60	60	12.5	11.3	5.6	60	81	14.1	12.0	5.8	45	123	14.1	11.6	5.6	65	165	14.1	11.4	5.4	65
60	M	3a	32	12.5			60	60	12.5	12.0	6.0	60	81	12.5	11.7	5.6	45	123	14.1	11.5	5.5	65	165	15.6	11.3	5.3	60

61	F	15	21					49	12.5	12.0	5.8	60	60	14.1	12.0	5.9	50	102	14.1	11.8	5.7	65	144	15.6	11.6	5.5	65
62	M	15	21					49	12.5	11.8	5.6	50	60	14.1	11.6	5.9	60	102	14.1	11.7	5.6	65	144	15.6	11.5	5.4	65
63	F	15	21					49	12.5	12.0	6.2	60	60	14.1	12.0	5.8	60	102	14.1	11.6	5.7	65	144	15.6	11.4	5.5	65
64	F	15	21					49	12.5	11.8	6.0	60	60	10.9	11.8	6.0	60	102	15.6	11.5	5.5	60	144	14.1	11.3	5.3	60
65	F	15	21					49	12.5	11.6	5.6	50	60	12.5	12.0	5.9	60	102	15.6	11.8	5.7	60	144	15.6	11.6	5.5	65
66	F	15	21					49	12.5	11.8	5.9	60	60	12.5	11.8	6.0	45	102	Used for study								
67	F	15	21					49	10.9	11.5	5.6	60	60	10.9	11.7	5.6	45	102	Used for study								
Sodium fluoride group																										
68	M	12	146	14.1	11.8	6.2	60	174	14.1	11.4	5.8	60	195	15.6	11.7	5.5	55	235	14.1	11.2	5.4	60	277	15.6	11.0	5.2	60
69	F	12	146	15.6	11.8	5.8	50	174	14.1	11.6	5.8	65	195	15.6	11.5	5.6	60	235	12.5	11.4	5.6	60	277	15.6	11.2	5.4	60
Deflucrophos group																										
70	M	16	19	—	—	—	—	47	12.5	12.6	6.2	50	68	12.5	12.0	5.8	45	Killed and used for study								
71	M	17	Unborn					Unborn					7	—	—	—	—	49	12.5	12.0	6.0	50	91	14.1	12.0	5.8	60
72	M	17	"					"					7					49	12.5	11.8	5.8	55	91	15.6	11.6	5.6	60
73	F	17	"					"					7					49	12.5	11.6	5.7	65	91	15.6	11.4	5.5	60
74	F	17	"					"					7					49	12.5	12.0	6.0	60	91	14.1	11.8	5.8	60
75	F	17	"					"					7					49	12.5	11.6	5.6	60	91	14.1	11.4	5.4	65
76	M	1a	"					"					Unborn					19	—	—	—	—	61	14.1	12.0	6.0	65
77	F	1a	"					"					"					19					61	14.1	11.8	5.8	65
78	M	1a	"					"					"					19					61	14.1	11.9	5.7	65
79	M	1a	"					"					"					19					61	14.1	12.0	5.9	60
80	M	1a	"					"					"					19					61	14.1	11.9	5.8	65
81	F	1a	"					"					"					19					61	14.1	12.0	6.0	60

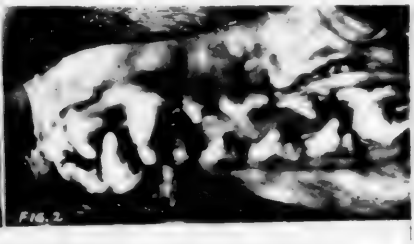
TABLE III

Fluorine content of femurs of dogs

DOG NO.	LITTER NO.	AGE	NO. OF DAYS GESTATION MOTHER HAD FLUORINE SUPPLEMENT	TYPE FLUORINE SUPPLEMENT ADDED TO BASAL RATION*	FLUORINE CONTENT OF FEMUR
		days			*p.p.m.***
8	1	1	None	None	40
16	1	47	"	"	5
21	1	58	"	"	41
22	1	58	"	"	35
25	1	68	"	"	97
26	1	78	"	"	63
28	10	123	"	"	123
30	10	127	"	"	175
42	11	194	"	"	258
1	2	2	26	Bone meal powder	61
82	21	2	62	"	51
2	2	9	26	"	63
17	3	23	20	"	12
18	3	24	20	"	93
83	21	26	62	"	37
87	21	27	62	"	25
19	3	30	20	"	65
23	3	35	20	"	63
4	7	49	26	"	60
6	2	53	26	"	87
66	15	97	62	"	230
67	15	97	62	"	191
58	3a	109	62	"	306
55	2a	110	62	"	310
35	16	2	41	Defluorophos	28
36	16	2	41	"	22
33	18	22	8	"	41
34	18	29	8	"	41
70	16	98	41	"	258
29	8	108	21	"	102
9	9	1	21	Sodium fluoride	39
10	9	4	21	"	80
11	9	4	21	"	125
12	9	4	21	"	80
13	9	4	21	"	74
14	9	4	21	"	61
15	9	5	21	"	64
3	4	23	37	"	110
24	9	27	21	"	54
5	4	41	37	"	132
27	12	44	None	"	378
7	4	48	37	"	290
31	12	102	None	"	2150
32	12	108	None	"	1920

* Supplements added at a level to give 5 mgm. of fluorine per kilogram of body weight daily.

** Parts per million.



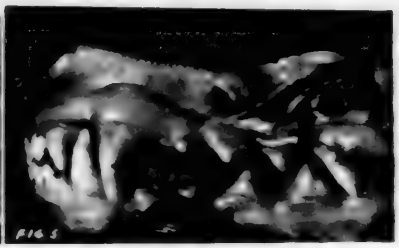


Fig. 1. Control animal which received basal ration for 435 days. Note hypoplastic defects in enamel of anterior teeth, and anterior position of mandibular incisors.

Fig. 2. Dog received basal ration plus low fluorine-containing U.S.P. $Ca_3(PO_4)_2$ for 414 days. Note hypoplastic defects in incisors and canines.

Fig. 3. Dog received basal ration plus sufficient defluorinated phosphate to provide 5 mgm. fluorine per kgm. body weight. Dog died of respiratory infection at 189 days of age. All teeth are well formed and entirely free from anatomic defects with minimum of superficial staining.

Fig. 4. Dog received basal ration plus sufficient bone flour to provide 5 mgm. of fluorine per kgm. body weight for 437 days. Teeth are well formed and free from anatomic defects with minimum of superficial staining.

Fig. 5. Dog received basal ration plus sodium fluoride to provide 5 mgm. fluorine per kgm. body weight for 387 days. Note definite areas of defective enamel and staining of enamel.

Several young dogs died during the early experiments due to respiratory infections and canine distemper. The use of appropriate vaccines and serums reduced the mortality in the subsequent experiments. The interval between the death

of the animals and autopsies was kept as short as possible and in many instances autopsies were performed at the time of death. Extensive histopathological studies are in progress and will be reported in a later communication. The bones (rib, sternum, and femur) were fixed in Zenker's solution (with 10% formalin) decalcified in a sodium citrate-formic acid solution, and the celloidin sections were stained with hematoxylin-eosin.

Table I gives a brief summary of the dogs used in the different experiments.

DISCUSSION OF RESULTS

At the beginning of the experiments, 2 young puppies fed 5 mgm. of fluorine per kilogram of body weight as bone meal powder and defluorinated phosphate

TABLE IV

Typical physical measurements of the femurs and tibias of dogs fed fluorine at five mgm. per kgm. of body weight as it occurs in purified bone meal and defluorinated phosphate

DOG NO.	SEX	BONE	AGE OF DOG AT END OF EXPERIMENT	LENGTH	SMALLEST DIAMETER	BREAKING STRENGTH	SPAN
Purified bone meal							
			days	*cm.*	*cm.*	*kgm.*	*cm.*
64	F	Femur	228	12.50	0.89	64.1	10
		Tibia		12.50	0.84	52.5	10
		Tibia		12.50	0.82	52.9	10
65	F	Femur	265	14.00	0.98	82.3	10
		Tibia		14.50	0.98	63.0	10
		Tibia		14.50	0.94	68.9	10
Defluorinated phosphate							
73	F	Femur	175	14.00	1.20	93.2	10
		Tibia		15.00	0.97	84.5	10
		Tibia		15.00	0.98	90.4	10
75	F	Femur	175	14.50	0.98	83.6	10
		Tibia		14.20	0.72	84.3	10
		Tibia		14.20	0.72	85.9	10

developed bowed legs which resembled rickets. The vitamin D content of the rations was increased from about 20 to 50 I.U. (International Units) and the legs became normal again. Roentgenograms of the legs of these puppies and others revealed a normal development. Several subsequent litters of puppies were raised on the same rations with normal bone development after the vitamin D content of the ration had been increased.

Michaud and Elvehjem concluded that with a normal calcium:phosphorus ratio of 1.2:1 a dose of 10 to 20 I.U. vitamin D per kilogram of body weight is an adequate daily supply for dogs. When the calcium:phosphorus ratio was increased to 2:1, bone calcification was very poor in the presence of limited amounts of vitamin D but improved as soon as the ratio was reduced.

The calcium and phosphorus ratio of the unsupplemented basal ration used in these studies was about 1.4:1. The calcium and phosphorus ratios in the purified bone meal and defluorinated phosphate were 2.2:1 and 2.7:1. When large

TABLE V

Growth of puppies whose mother received basal ration during pregnancy and lactation

Litter No. 10. (Mother's weight ca. 12 kilograms)

AGE	PUP NO.			
	28	30	44	43
	Sex			
	M	F	M	M
	Weight			
days	*kgm.*	*kgm.*	*kgm.*	*kgm.*
24	1.25	0.99	1.10	1.34
35	1.58	1.33	1.28	1.82
42	1.58	1.59	1.27	2.21
49	1.62	1.70	1.28	2.48
56	2.08	2.07	1.64	2.89
63	2.53	2.32	2.00	3.45
70	3.08	2.63	2.25	3.88
77	3.40	3.02	2.82	4.49
84	3.95	3.30	3.40	4.90
91	4.05	3.65	3.60	5.40
98	4.00	3.60	3.60	5.50
105	3.40	4.00	3.80	5.90
112	Died, used for	4.00	4.20	6.00
119	study	Died, used for	4.40	6.40
126		study	4.80	6.55
133			5.00	7.20
140			5.30	7.70
147			5.60	7.90
154			5.70	8.10
161			5.90	8.30
168			6.20	9.10
175			6.50	9.50
182			6.60	9.60
189			6.90	9.90
196			6.90	10.40
203			7.00	10.50
210			7.10	10.60
217			7.40	10.90
224			7.50	11.40
231			7.50	11.40
238			7.50	11.40
245			7.50	11.40

quantities of these supplements were added to the basal ration, the calcium: phosphorus ratio was increased and larger amounts of vitamin D were required to produce normal bone development.

Blood analyses were made on the adult dogs and on puppies at different periods during the calcification and eruption of their deciduous and permanent teeth. Data on the blood of the dogs are recorded in Table II. The concentrations of hemoglobin, serum calcium and phosphorus, and coagulation time are within the normal range reported for this species. The hemoglobin content of the blood

TABLE VI

Growth of puppies whose mother received 5 mgm. of fluorine (as bone meal powder) per kilogram of body weight during last 35 days of pregnancy and lactation

Litter No. 13. (Mother's weight ca. 10 kilograms)

AGE	PUP NO.					
	48	49	50	51	52	53
	Sex					
	M	F	F	F	F	M
	Weight					
days	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*
Birth	0.32	0.39	0.33	0.33	0.31	0.38
3	0.41	0.43	0.40	0.38	0.37	0.43
10	0.70	0.70	0.63	0.60	0.63	0.90
17	0.80	0.75	0.77	0.70	0.70	1.00
24	1.00	0.80	0.90	0.85	0.79	1.20
31	1.28	1.90	1.02	1.05	0.86	1.44
38	1.69	1.14	1.30	1.38	1.07	1.89
45	2.22	1.48	1.65	1.72	1.41	2.41
52	2.70	1.66	1.80	1.92	1.66	2.84
59	3.38	2.44	2.43	2.50	2.10	3.45
66	3.85	2.65	2.71	2.85	2.65	4.00
73	4.05	2.70	3.05	3.20	2.85	4.40
80	4.60	3.40	3.40	3.60	2.90	4.90
87	5.00	3.50	3.80	3.90	3.40	5.30
94	6.00	4.00	4.20	4.60	3.60	6.20
101	6.10	4.10	4.30	4.70	3.70	6.30
108	6.42	4.00	4.50	4.74	3.60	6.08
115	7.00	4.70	5.20	5.10	4.30	6.80
122	7.40	4.90	5.30	5.60	5.00	7.40
129	7.60	5.20	5.80	5.90	5.50	7.80
136	7.80	5.40	5.80	5.90	5.50	8.00
143	7.90	5.80	6.20	6.40	6.00	8.80
150	8.50	6.20	6.60	6.70	6.20	9.20
157	8.80	6.30	6.60	6.80	6.20	9.20

Puppies weaned at 59 days of age.

increased as the puppies grew older but the serum calcium and phosphorus declined slightly. This is in agreement with previous studies on dogs (11).

The fluorine content of the dry-fat-free femurs of some of the dogs is summarized in Table III. These data indicate that there was a gradual increase in the fluorine content of the femurs as the animals grew older. The dogs fed 5 mgm. of fluorine as sodium fluoride stored much higher concentrations of fluorine than

puppies receiving the same level of fluorine as bone meal powder or defluorinated phosphate. The permanent teeth of the puppies fed the above level of fluorine as sodium fluoride showed evidence of dental fluorosis, but puppies that received

TABLE VII

Growth of puppies whose mother received 5 mgm. fluorine as bone meal powder per kilogram of body weight during growth, pregnancy and lactation

(Second generation on bone meal powder)

Litter no. 21 (Mother's weight ca. 10 kilograms)

AGE	PUP NO.						
	82	83	84	85	86	87	88
	Sex						
	F	M	M	M	M	M	M
	Weight						
days	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*
Birth	0.25	0.26	0.26	0.26	0.26	0.25	0.26
7	Used for	0.39	0.39	0.32	0.45	0.35	0.37
14	study	0.43	0.52	0.42	0.55	0.43	0.47
21		0.65	0.75	0.52	0.75	0.48	0.58
28		Used for	0.93	0.72	0.85	Used for	0.77
35		study	1.13	0.90	1.02	study	1.05
42			1.42	1.03	1.20		1.41
49			1.86	1.26	1.65		1.85
56			2.20	1.54	2.01		2.15
63			2.60	1.84	2.50		2.60
70			2.80	2.04	2.80		2.85
77			3.30	2.30	3.10		3.25
84			3.90	2.50	3.30		3.60
91			4.40	3.20	3.80		4.20
98			4.60	3.50	4.20		4.80
105			Used for	Used for	4.40		5.20
112			study	study	5.00		5.60
119					5.20		5.80
126					6.00		6.20
133					6.60		6.60
140					7.00		7.00
147					7.10		7.30
154					7.20		7.80
161					7.40		8.40
168					7.70		8.30
175					7.80		8.10
182					7.80		8.10

the same level of fluorine as it occurs in bone meal powder and defluorinated phosphate had sound, normal teeth (*figs. 1–5*).

Physical measurements, including breaking strength tests, were made after death on the femurs and tibias of representative dogs fed the bone meal powder and defluorinated phosphate for about 1 year. The breaking strength tests were

made on an Olsen dyanometer at the Armour Laboratories through the cooperation of C. A. Fischl and W. R. Savage. In all tests, the bones were supported at the ends and loaded at the midspan. The breaking strength data indicated that the bones were in the normal range. Typical data on the physical

TABLE VIII

Growth of puppies whose mother received 5 mgm. of fluorine as defluorophos per kilogram of body weight during last 7 days of pregnancy and during lactation

Litter No. 17. (Mother's weight ca. 10 kilograms)

AGE	PUP NO.				
	71	72	73	74	75
	Sex				
	M	F	M	F	F
	Weight				
days	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*
Birth	0.28	0.26	0.26	0.27	0.29
7	0.45	0.44	0.36	0.44	0.40
14	0.90	0.85	0.86	0.94	0.82
21	1.24	1.19	1.32	1.20	1.12
28	1.65	1.55	1.69	1.59	1.53
35	2.00	2.10	2.10	1.93	1.90
42	2.70	2.55	2.65	2.40	2.40
49	3.35	3.00	3.00	2.90	2.80
56	4.30	3.80	3.20	3.20	3.20
63	4.60	4.20	4.20	3.90	4.00
70	4.80	4.80	4.50	4.00	4.10
77	5.40	5.20	5.00	4.70	4.60
84	5.60	5.60	5.20	4.80	4.80
91	6.20	6.00	5.50	5.00	5.00
98	6.40	6.20	5.70	5.20	5.20
105	7.20	6.60	6.10	5.80	5.60
112	7.80	6.90	6.40	6.20	6.00
119	8.00	7.20	6.60	6.60	6.40
126	8.40	7.60	6.80	6.80	6.80
133	8.60	7.80	6.90	Used for study	6.80
140	8.80	8.10	7.20		7.00
147	9.00	8.20	7.50		7.10
154	Used for study	8.30	7.60		7.20
161		8.80	7.90		7.30
168		9.20	8.10		Used for study
175		9.40	Used for study		
182		9.50			

Puppies weaned at 61 days of age.

measurements are given in Table IV. These results are in agreement with previous studies (12) on dogs.

Data relating to the growth of 1 litter of pups from each group fed the basal ration, and the basal ration supplemented with bone meal powder, Defluorophos,

TABLE IX

Growth of puppies whose mother received 5 mgm. of fluorine as sodium fluoride per kilogram of body weight during lactation

Litter No. 12. (Mother's weight ca. 10 kilograms)

AGE	PUP NO.				
	27	31	32	68	69
	Sex				
	M	M	F	M	F
	Weight				
days	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*
Birth	0.32	0.27	0.24	0.26	0.25
7	0.50	0.48	0.37	0.42	0.44
14	0.71	0.71	0.63	0.63	0.66
21	0.90	0.96	0.77	0.82	0.71
28	1.13	1.21	1.03	1.10	0.93
35	1.24	1.43	1.24	1.41	1.05
42	1.31	1.70	1.31	1.31	1.22
49	1.60	1.90	1.60	1.60	1.40
56	Used for study	2.30	1.80	1.70	1.50
61		2.80	2.00	2.00	1.90
68		3.20	2.10	2.20	2.00
75		3.60	2.30	2.60	2.50
82		3.90	2.30	3.20	2.90
89		3.80	2.50	3.80	3.40
96		3.40	2.20	4.10	3.80
103		Died, used for study	2.30	4.50	4.20
110			Died, used for study	4.70	4.40
117				5.40	5.10
124				5.70	5.20
131				6.10	5.80
138				6.50	5.90
145				7.00	6.20
152				7.60	6.60
157				7.90	6.70
164				8.00	7.20
171				8.10	7.20
178				8.30	7.40
185				8.40	7.40
192				8.50	7.60
208				9.40	8.00
219				9.50	8.20
226				10.20	8.30

and sodium fluoride are recorded in Tables V to IX. Of special interest is the growth of puppies described in Table VII. This litter represents the second generation of dogs to receive the 5 mgm. of fluorine as bone meal powder per kilogram of body weight. The growth, bone and tooth development appeared normal.

Preliminary studies of the bones of representative animals in all the groups at varying ages up to 140 days have been completed. Grossly, there were no deformities or changes in the texture or color of the bones of the calvarium, rib, sternum or femur.

Changes of increased rate of subperiosteal bone production and secondary resorption of compact bone such as those reported in sows by Kick, Bethke, Edgington, Wilder, Record, Wilder, Hill, and Chase (15), or of matrix granularity, and osteocytic pyknosis reported by Sutro (25) in rats fed high concentrations of sodium fluoride, or the malformation and increased bone development or similar changes as described by Largent, Machle and Ferneau (17) at higher levels of fluoride intake have not been observed. Bauer (1a) has recently described sclerotic periosteal and endosteal bone proliferation in dogs fed higher concentrations of fluorine than were used in this study. Such changes have not been present in our preliminary material. The epiphyseal lines were regular and well formed with no increased fibrosis or rarifaction. The Haversian systems were well developed and the osteocytes appeared viable and unchanged. Normal osteoblastic and osteoclastic activity incident to normal growth was evident but not increased. The bone marrow showed the usual amount of myelopoietic and erythropoietic activity. Young puppies of the second fluorine-fed generation whose mothers received fluorine during gestation and lactation showed normal active marrows. Older dogs which died of acute infections exhibited hyperplastic marrows with no histologically demonstrable impairment of the usual myelopoietic response.

The teeth of all of the dogs were examined with the aid of a mouth mirror and explorer under good lighting conditions at frequent intervals. The dogs serving as controls and, therefore, on either the basal ration or the basal ration plus calcium phosphate had teeth of normal contour and color. Several areas of enamel defects in the form of pits were diagnosed as hypoplasia of the enamel. Some of the teeth of the control animals exhibited areas of abrasion, and in all cases a rather heavy deposit of calculus was found on the fourth premolar and the first and second molar. This deposit was a greenish-grey color and clinically was comparable to salivary calculus found in the mouths of humans.

The 20 animals fed the bone meal supplement for varying periods up to 518 days had teeth which were entirely free from hypoplastic defects, of a normal color, and had unusually clean mouths. Only 2 animals had deposits of calculus and this was small in amount. Eleven dogs fed the Defluorophos supplement for varying periods of time up to 249 days had a dentition of excellent quality. Their mouths were unusually clean; only 1 dog had a small amount of calculus on the posterior teeth. There was no evidence of hypoplastic defects of the enamel, areas of bleaching, or even the faintest staining of the enamel.

Two dogs which had reached 429 days of age and had been fed the sodium fluoride supplement exhibited teeth that were stained a light yellow and had many hypoplastic defects or pits in the enamel, especially in the anterior teeth. In some areas the enamel appeared soft and chalky and could readily be removed with the scaler. One of these animals had an end-to-end relationship of the anterior teeth and, therefore, exhibited some abrasion in the anterior region.

The accompanying illustrations (*figs. 1–5*) depict the dental conditions of animals taken from each of the foregoing groups. It was clearly evident to all who observed these dogs at each examination that those dogs which were fed either bone meal or Defluorophos supplements revealed a better dentition and a cleaner mouth than the control animals.

SUMMARY

1. Studies on 21 litters of dogs have revealed there was no significant alteration in serum calcium and phosphorus, hemoglobin, blood coagulation time and growth in dogs fed fluorine as it occurs in sodium fluoride, purified bone meal powder, and defluorinated phosphate at a level of 5 mgm. per kilogram of body weight daily, up to a period of about 1 year, under these experimental conditions. Chronic studies covering longer periods of time are in progress.

2. Puppies fed 5 mgm. of fluorine as sodium fluoride per kilogram of body weight daily during the period of calcification of their teeth developed a dental fluorosis and stored excessive fluorine in their bones, while other puppies fed the same level of fluorine as it occurs in purified bone meal powder and defluorinated phosphate did not develop dental fluorosis or store excessive fluorine in their bones. Bone development appeared normal in all groups as revealed by breaking-strength tests, roentgenograms, and histological studies. These studies are still in progress. Their body growth was not impaired.

3. The teeth of 20 dogs fed 5 mgm. of fluorine per kilogram of body weight daily as bone meal powder and 11 dogs fed defluorinated phosphate were superior to the teeth of dogs fed the same level of fluorine as sodium fluoride or the control ration supplemented with about the same level of calcium and phosphorus as found in the bone meal powder or defluorinated phosphate.

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THE EFFECT OF SODIUM FLUORIDE ON THE TEETH AND BLOOD CALCIUM AND INORGANIC PHOSPHORUS LEVELS OF RACHITIC RATS[1]

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In a previous paper (Irving, 6), it was shown that the action of fluorine on the teeth was governed to a large extent by the Ca:P ratio of the diet. With normal diets, the injection of NaF caused the changes already well known and described by Schour and Smith (13). With diets of high (4.4) or low (0.29) Ca:P ratio, teeth were produced with wide predentin and looking histologically indistinguishable. NaF had no effect on the dentin of the teeth of animals on the high Ca:P ratio diet till the predentin laid down at the time of injection was calcified, which might take several days instead of the usual 24 hours. In the case of animals on the low Ca:P ratio diet, however, a fine calcified line appeared 12 hours after the NaF injection in the predentin which was being laid down at the time of injection. Irving explained these differences in reaction by supposing that F acted primarily by altering the composition of the blood, since the blood Ca and inorganic P values are very different in animals made rachitic with high and low Ca:P ratio diets. The experiments quoted in the present paper were undertaken to investigate the changes in blood composition under these various conditions.

EXPERIMENTAL

Diets. The 3 diets used had the same constituents as previously (Irving, 6). The batches used during the present experiment gave on analysis the following figures for Ca and P:

Diet no.	Ca %	P%	Ca:P
1	1.23	0.84	1.5
2	1.31	0.24	5.5
3	0.098	0.37	0.26

Diet 1, which was the stock diet of the rat colony, was prepared in cubes and fed as such. Diets 2 and 3 were given as a powder slightly moistened with water.

Rats. The animals used were Wistar Institute strain albino rats, housed under the same conditions as before. Males and females were used indiscriminately, the litter size varying from 6 to 9. After weaning at 23 days of age, they were given the stock diet ordinarily used. In the case of diets 2 and 3, the rats were transferred to these diets when between 50 and 60 g. weight, and kept on them for

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28 days. They were then injected once with saline or NaF solution. The rats on diet 1 were injected in the same way when they were about 70 g. in weight, as this was the usual weight of rats after 28 days on diets 2 or 3. 6 litters of rats were used on diet 1, 10 litters on diet 2 and 12 litters on diet 3.

Injections. All injections were made subcutaneously. For the fluoride injections, 2% NaF was used. The animals on diets 1 and 2 were given about 26 mg. F per kg. body weight, which was an injection volume of about 0.2 cc. This was fatal in only a few instances, the animals going into a comatose condition before death. The animals on diet 3, as had been previously found (Irving, 6) rapidly went into tetany and succumbed at this dose level, and the dosage used was reduced to about 9 mg. F per kg. body weight, an injection volume of about 0.07 cc. Convulsive seizures, not always fatal, occurred at times at this dose level. In each group, 2 or 4 litters acted as controls and were given the same volume of 0.9% NaCl solution. After NaF injections, the animals will not usually eat for 24 hours, and the controls were therefore starved after the saline had been given.

One animal of a litter was killed at the time of injection and the rest were planned to be killed at 3, 6, 9, 12, 18, 24, 30 or 36 hours subsequently, depending on the size of the litter. In the case of animals dying prematurely, the rest were all moved up 1 period so that the early part of the time-table could be carried through without a break. 4 litters on diet 1, 6 litters on diet 2, and 8 litters on diet 3 were injected with NaF. Two litters on diet 1, 4 litters on diet 2 and 4 litters on diet 3 were injected with saline.

Methods of analysis. The animals were anaesthetized with ether and mixed arterial and venous blood collected. Ca estimations were carried out by the method of de Loureiro and Janz (4) on the blood serum, and the inorganic P of the whole blood was estimated by the method of Youngburg and Youngburg (16) modified for use with a photoelectric colorimeter. As it was impossible to obtain enough blood for both estimations from one animal, Ca estimations were made in the case of half the litters used, and P was estimated in the blood of the rest of the rats. The skulls were placed in formol-saline solution and histological preparations were made of the upper incisor teeth by the methods previously described (Irving, 6). The teeth of 2 litters on each diet were examined, particular note being taken of changes on the labial side.

RESULTS

The rats on diet 1 were in all respects normal healthy animals. The rats on diet 2 all had severe rickets. Those on diet 3 did not have rickets but animals on this type of diet have a considerable reduction in the bone ash (Irving, 7) and degree of calcification of the bone (Boyle and Wesson, 2). The calcification of the teeth of animals on diet 2 and 3 was considerably impaired.

Diet 1. Teeth. The same histological changes as previously described (Schour and Smith, 13; Irving, 16) were found, but appeared somewhat sooner than previously reported by Irving, 9 hours after injection in one litter, and at 12 hours in the other.

Blood. The Ca and P values are shown in Table I. In spite of the pronounced histological changes, it will be seen that the serum Ca and blood P were unaffected by the NaF injections. The Ca values of the control litter were higher and the P values lower than normal but they remained fairly constant over the period of the experiment.

TABLE I

Serum Ca and blood inorganic P values (mg. per 100 cc.) of starved rats on diet 1, injected with NaF solution or saline

HOURS	SERUM Ca			BLOOD INORGANIC P		
	NaF		Saline	NaF		Saline
	Litter number					
	813	811	828	810	818	850
0	9.1	10.3	13.0	8.5	9.4	5.8
3	8.9	10.0	11.9	10.1	8.5	5.6
6	9.1	10.2	12.1	10.5	8.4	7.0
9	9.3	9.6	13.3	9.6	7.4	6.3
12	9.6	10.2	11.5	11.0	7.0	6.4
18	9.6	—	11.2	13.5	8.2	6.0
24	10.3	—	12.0	8.9	8.1	6.2
30	11.9	—	11.3	8.5	—	5.7
36	—	—	12.8	10.5	—	—

TABLE II

Serum Ca and blood inorganic P values (mg. per 100 cc.) of starved rats on diet 2, injected with NaF solution or saline

HOURS	SERUM Ca					BLOOD INORGANIC P				
	NaF			Saline		NaF			Saline	
	Litter number									
	765	766	779	791	839	781	787	798	793	837
0	10.2	10.4	10.8	10.3	12.2	1.4	3.0	1.5	—	2.1
3	8.7	8.7	8.4	9.9	11.6	2.3	2.2	2.6	1.6	2.4
6	9.5	8.5	6.0	9.2	9.2	3.7	4.7	5.4	2.7	3.5
9	6.4	8.3	6.0	10.3	9.3	4.6	4.4	3.9	2.7	3.8
12	6.8	6.5	6.7	10.0	10.0	4.9	5.8	5.0	3.0	3.3
18	5.9	6.1	—	9.3	5.6	5.4	6.4	5.5	3.8	4.0
24	—	—	7.1	6.1	6.8		4.6		3.9	4.5
30	5.8	—		7.6	—				5.0	4.7

Diet 2. Teeth. As was previously found, no changes at all occurred in the very wide proximal predentin over the period of the experiment; the distal dentin began to show changes similar to those of rats on diet 1, 18 hours after injection.

Blood. When animals on this type of diet are starved, the blood Ca falls and the inorganic P rises (Cavins, 3; Wilder, 15; Kramer, Shear and Siegel, 9). This

is well seen in the figures from the control rats (Table II). By 18–24 hours the blood Ca had fallen to about half its original values, and the P had begun to rise after 6 hours' starvation. It is an interesting point that the P had risen by 6 hours but the Ca remained within normal limits till 12 or 18 hours and had only

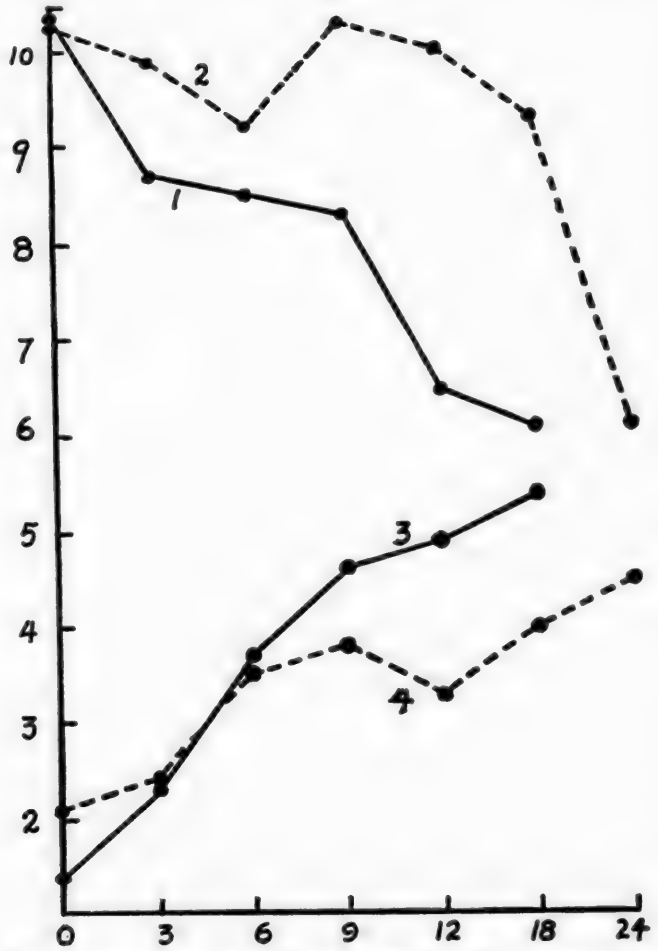

Fig. 1. Blood inorganic P and serum Ca levels of rats on diet 2 injected with NaF or saline and starved. 1. Serum Ca of litter 766, injected with NaF. 2. Serum Ca of litter 791, injected with saline. 3. Blood inorganic P of litter 781, injected with NaF. 4. Blood inorganic P of litter 837, injected with saline.

fallen significantly after that time. The figures of Kramer *et al* (9) showed the same, the blood P almost doubling itself after 8 hours' fasting, whereas the Ca had not shown a significant fall till 21 hours had elapsed. The effect of NaF was to accelerate the blood changes. The Ca began to fall at once and continued to

do so more or less consistently, reaching quite low figures by 18 hours. The P also rose soon after the injection, doing so at a faster rate than in the starved

TABLE III

Serum Ca and blood inorganic P values (mg. per 100 cc.) of starved rats on diet 3, injected with NaF solution or saline

HOURS	SERUM Ca						BLOOD INORGANIC P					
	NaF				Saline		NaF				Saline	
	Litter number											
	736	780	786	869	792	844	743	723	899	896	796	840
0	5.6	4.8	5.0	5.6	4.3	6.4	6.9	—	7.0	6.7	5.7	6.0
3	3.8	4.0	3.5	3.7	3.9	5.0	5.7	8.1	8.7	13.7	5.8	7.1
			4.0									
6	—	4.4	4.6	4.4	3.9	5.8	6.0	6.5	9.1	7.8	5.4	6.7
9	3.8	—	3.9	3.3	3.3	5.4	6.9	6.0	8.1	7.9	4.6	6.3
12	—	4.8	2.9	4.5	4.6	5.0	7.8	6.2	—	7.2	6.0	6.4
18	—	3.5	—	4.4	4.6	—	8.9	7.2	—	—	7.0	6.4
								6.7				
24	—	—	4.3	—	—	6.4	—	7.7	—	—	6.4	6.9
30	—	—	—	—	—	7.0	—	—	—	—	—	7.1
36	—	—	—	—	—	—	—	—	—	—	—	6.4

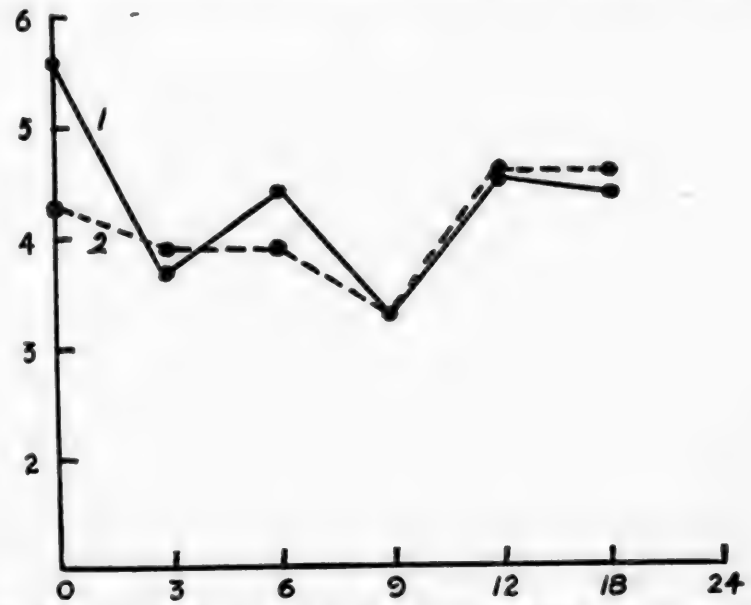

Fig. 2. Serum Ca levels of rats on diet 3, injected with NaF or saline and starved. 1. Litter 869, injected with NaF. 2. Litter 792, injected with saline.

animals. The changes over 24 hours in 2 litters injected with NaF and 2 control litters are also shown in *fig. 1*.

Diet 3. Teeth. The same results as previously reported (Irving, 6) were found. The fine hypercalcified line caused by NaF in the predentin was first seen 9 hours after the injection. From measurements of its width, calculation showed that the NaF had begun to act at once and had acted for from 5.3 to 5.5 hours.

Blood. The blood Ca began to fall at once in the animals injected with F and remained at a lower level for some hours (Table III). The blood Ca also fell, though to a less degree, in the control rats. A large number of the animals injected with NaF had attacks of tetany, whereas none of those injected with saline were affected in this way. Thus it would seem that the differences in depression of the blood Ca in the two groups, though slight, was a real one. The blood inorganic P figures of the rats injected with NaF and with saline showed considerable variations in both directions. The one animal with a P value of 13.7 mg. per 100 cc. serum was killed at the time of a tetanic seizure. Serum Ca figures of a litter injected with NaF and of a control litter are shown in *fig. 2.*

DISCUSSION

This work was originally undertaken to see if fluoride exerted its effects upon the teeth by altering the composition of the blood, as had been suggested by Irving (16). Schour and Smith (13) considered that there was a direct action upon the tooth.

In the case of the action of F upon the formation of enamel the suggestion of Schour and Smith is probably true, since the same changes are seen in the organic enamel of the teeth of rats on all of the three diets. Irving's original hypothesis was based on the following findings, which are confirmed in the present results: with both diets 1 and 2, the changes in the teeth were preliminary to calcification of the predentin to dentin; with diet 1 they occurred about 18 hours after injection, with diet 2, the time of appearance depended on the width of the predentin. With diet 3, however, a fine hypercalcified line was seen in the predentin 9 to 12 hours after injection. This was not a precalcification phenomenon but a form of new calcification. In all cases, measurements showed that the predentin being formed at the time of injection was affected by the F, and retained a potentiality for abnormal calcification which became evident later. Since the only differences in the rats on diet 2 and 3, whose teeth appeared identical histologically, was in the Ca:P ratio of the diet and thus in the blood Ca and P levels, it was not unnaturally concluded that the F exerted its action by altering the blood Ca and P values.

The present results show that with diet 1, in spite of the marked histological changes in the teeth, F had no effect upon the blood Ca and P values. This result is not unexpected, as in normal animals many influences operate to keep the blood Ca and P steady. Thus the ingestion of Ca salts has a very small effect upon the blood Ca level in normal animals. The administration of 50 mg. of Ca as $CaCl_2$ per kg. body weight to dogs caused, after 2 hours, a rise of at most 1 mg. Ca per 100 cc. serum (Freeman, Kant and Ivy, 5). The F injected in the present experiments had, in the case of animals on diet 1, a Ca combining power of about 26

mg. per kg. body weight. If the results of Freeman, Kant and Ivy can be applied reversibly, the administration of this amount of F would cause a fall of less than 1 mg. Ca per 100 cc. serum. In addition, the trabeculae of the bones of animals on diet 1 would be well filled and a good reserve for extra Ca.

In the case of animals on diets 2 and 3, F caused an immediate fall in blood Ca. In these rats, the bone ash was lowered and the amount of Ca reserve in the trabeculae much less than in the rats on diet 1. Irving (6) showed that F caused a change in the predentin laid down at the time of injection, which later caused abnormal calcification. One is forced to conclude that the two phenomena are in some way connected. The immediate supposition would be that CaF_2 had been deposited in the tooth, but against this is the fact, previously stressed by Irving (6), that no hypercalcification, at least as demonstrated histologically, is visible till several hours later. This may not be an insuperable objection, as the CaF_2 may not be in a form readily stainable, but normally predentin is considered not to contain any of the elements of calcification. However, in favour of this view are the findings of Irving (8) that while both citrate and phosphate produced tetany when injected into animals on diet 3, only phosphate caused the formation of a hypercalcified line in the predentin similar to that following F. The citrate would be metabolised and disappear, but the P could presumably be laid down in the predentin as a Ca salt in the same way as F. In addition Volker, Sognnaes and Bibby (14) and McClendon and Foster (11) have shown that the F content of the tooth rises when F is administered. If this conception is true, the action of F would be a variant of the normal process of calcification.

While the general action of F upon the tooth seems explicable on the above terms, it is not yet possible to see why the teeth of rats on diet 2 and 3 responded so differently. It may be that with a low blood Ca the conditions for precipitation of CaF_2 are sooner satisfied and the action is quickly over, lasting about 5 hours; while with a high blood Ca, the action may take longer to come on. Or a different kind of tooth may be produced with the two different diets. This view is supported by the fact that the response of the dentin to vitamin D is quite different when the animal has been on a low Ca:P ratio diet from what it is when the dietary Ca:P ratio is high (Irving, 7).

It is well known that in many conditions the blood Ca and P values bear an inverse relationship to each other, but it seems doubtful that the levels mutually govern each other through physicochemical relationships operating in the blood. Thus Logan and O'Connor (10) suggested that after parathyroid administration, the rise in Ca was due to solution of bone, the fall in P being caused by active secretion by the kidney. The animals on diet 2 which were injected with saline and starved, showed the usual reciprocal changes, explicable on the hypothesis of McLean and McCoy (12), that colloidal calcium phosphate is formed in the blood and promptly removed. In the animals injected with F, the Ca fell at once, and the P rose more promptly than in the starved rats. It was suggested earlier that the fall in Ca was due to the deposition of CaF_2 in the calcifying structures. The quick rise in P is not explicable at first sight. It may possibly be due to a toxic action of F either on the kidneys or on the liberation of P from the soft tissues.

The P values of the rats on diet 3 are also somewhat puzzling. The changes

in all the other blood figures agreed among the litters examined, but here the results were quite inconsistent. A fall in the blood Ca at this low level should be accompanied by a large rise in P (Albright, Bauer, Cockrill and Ellsworth, 1) if the constancy of the reciprocal relationship of blood Ca and P invariable existed. One rat killed during tetany did have a very high blood P, possibly associated with a very low Ca value, but many of the P figures showed no rise when the blood Ca level was falling, and if, in fact, the P values are averaged, the resulting figures are remarkably constant. This appears to be another of the cases in which the reciprocity in the levels of blood Ca and P does not hold; and one must also conclude that F has no effect upon the blood P level under these conditions.

CONCLUSIONS

1. The effect of 1 subcutaneous injection of NaF solution upon the serum Ca and blood inorganic P of rats has been followed, and compared with the changes produced in the incisor teeth. Three groups of animals were used:

a. Normal rats,

b. Rats which had been on a diet with a high Ca:P ratio for 28 days, and

c. Rats which had been on a diet with a low Ca:P ratio for 28 days.

A certain number of control animals in each group were injected with saline and starved.

2. The blood Ca and P of the normal rats were unaffected by F.

3. The blood Ca of the rats on the high Ca:P ratio diet was initially within normal limits. It began to fall immediately after injection of NaF. The blood P was initially low, and started to rise immediately F was given. In the control animals, the usual starvation effect with rachitic animals was seen: the blood Ca remained steady for about 12 hours and then fell to low levels; the P began to rise at once, but more slowly than in the rats injected with NaF.

4. The blood Ca of the rats on the low Ca:P ratio diet was much lower than normal and fell still further after injection of F. Several of the rats had tetany. The P was initially higher than normal, and F had no consistent effect upon it. In the control animals, the Ca fell slightly, but the P remained fairly steady.

5. From a comparison of the histological changes in the teeth with the chemical findings in the blood, it was concluded that CaF_2 was probably deposited in the dentin by a variant of the normal process of calcification, and that this accounted for the histological changes observed. No explanation was forthcoming of the differences in response of the pre-dentin of animals on diets of high and low Ca:P ratio, previously reported by Irving.

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THE EFFECT OF CADMIUM ON THE PRODUCTION OF CARIES IN THE RAT[1]

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In the course of a study of chronic cadmium poisoning in the rat, Wilson, de Eds and Cox (1) noted that one of the first symptoms was the loss of the normal orange brown pigment of the incisor enamel. Such bleaching of the pigment had long been known to result from chronic fluoride poisoning. Although other conditions also produce this effect, it seemed possible that cadmium, since it is a phosphatase poison like fluorine, might have a similar effect on caries.

In accord with this idea, Ginn and Volker (2) fed rats that were on a caries producing diet 0.005% cadmium chloride in both food and drinking water. The cadmium in the food had no observed effect on caries, but the cadmium in the water produced an unexpected increase in the caries index. The authors did not suggest a mechanism for this effect, although they pointed out that the bleaching of the incisor pigment was probably due to interference with iron metabolism, since feeding cadmium was accompanied by a marked decrease in hemoglobin in the blood.

EXPERIMENTAL

As part of a general investigation of the effect of phosphatase poisons on the teeth, it seemed desirable to study the action of cadmium in greater detail. For this purpose, 3 groups of rats were placed on the Hoppert-Webber-Caniff coarse corn diet at 21 days of age. The first group received drinking water containing 0.002% cadmium chloride, the second received water containing 0.004% cadmium chloride, and the third served as controls. The diets were maintained for 100 days after which the animals were sacrificed and the teeth examined for caries by the technique of clearing in liquified phenol (3, 4). The caries index was expressed according to the method of Dale and Powell (5), so that the results could be compared directly with those of Ginn and Volker, who used this method. The results are shown in Table I. Since the rats given 0.004% cadmium chloride in drinking water showed a greater response in the sense of a wider range of the caries index, this concentration was used in subsequent experiments.

In the next series of experiments, 3 groups were used. One group of mothers was placed on 0.004% cadmium chloride from 2–6 days before the birth of the

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young, and the litter was maintained, with the mother, on this intake until the young were 23 days old, when calcification of the molars is complete (6). This group was then removed from the cadmium and, like the controls, given tap water, while on the caries producing diet for 100 days. The second group was treated in the same way as the first, except that administration of cadmium was continued during a 100 days period on the caries producing diet. The third group of controls received no cardmium. The results of this experiment are given in Table II.

The teeth in this experiment were analyzed for cadmium by the spectrophotometric method of Sandell (7), which will determine as little as 3γ of cadmium in 5 cc. of solution. The same teeth which were used in determining the caries index were submitted to analysis, illustrating another of the advantages of the

TABLE I

EXPERIMENTAL GROUP	NO. OF RATS	CARIES INDEX AVERAGE	CARIES INDEX RANGE	NO. OF RATS WITH NO CARIES
Ginn and Volker				
Controls	18	12.1	—	—
150 days on 0.005% $CdCl_2$	18	34.9	—	—
Leicester				
Controls	13	11.7	4–19	0
100 days on 0.002% $CdCl_2$	15	12.8	6–19	0
100 days on 0.004% $CdCl_2$	16	11.0	0–23	1

TABLE II

EXPERIMENTAL GROUP	NO. OF RATS	CARIES INDEX AVERAGE	CARIES INDEX RANGE	NO. OF RATS WITH NO CARIES
Controls	15	2.0	0–9	8
0.004% $CdCl_2$ during calcification	13	2.8	0–10	7
0.004% $CdCl_2$ during calcification and for the 100 day experimental period	18	4.2	0–12	6

liquified phenol technique. All the molars from a given rat were pooled, dissolved in 5% HCl, filtered and analyzed, resulting in an average figure of the cadmium in all the molars of each rat. The teeth of the rats in the control group and in the group which received cadmium during the calcification period only, did not give even a qualitative test for cadmium under these conditions. The teeth of the rats receiving cadmium throughout the whole experiment had an average content of 0.003% cadmium (range 0.001–0.0055%).

DISCUSSION

The results of these experiments are not as decisive as those of Ginn and Volker, probably because of the shorter period during which the diet was fed.

However, the results indicate a definite effect of cadmium. In the first experiment, the range of the caries index of rats given the higher concentration of cadmium was much greater than that of the controls. Since the number of carious lesions was no greater, the progress of the lesions was evidently more rapid in many cases. This fact would explain the greater caries score found by Ginn and Volker after 150 days.

The results in Table II are somewhat confused by the much lower caries indexes found, even in the controls. This was probably due to a change in the corn used in the caries producing diet. Such changes have been observed before in this laboratory, and seem to be due to a softer corn than usual. The possibility of a higher concentration of fluorine in the new corn is slight, since the control rats developed the normal orange pigment of the incisors on this diet. In any case, the general picture is still the same.

When cadmium was fed only during the calcification period, it showed practically no effect on the caries production by the experimental diet. The chemical analysis showed that this was because cadmium was not taken up by the tooth under these conditions. On the other hand, when cadmium was fed during the administration of the caries producing diet, it entered the tooth to a small extent, and results comparable to those in the first experiment were obtained.

Thus, there is a definite relation between the presence of cadmium in the teeth and the increased rate of development of caries, although the number of lesions does not appear to be increased. The lack of any connection between the bleaching of the rat incisor and the effect on caries is also demonstrated, since fluorine decreases caries and cadmium increases it, yet both bleach the incisors. It is worth noting that iodoacetate, which also reduces caries, has been observed in the course of other experiments in this laboratory to have no effect on the incisor pigment.

Ginn and Volker concluded from their experiments that the action of fluorine was probably unrelated to inhibition of lactic acid fermentation by oral organisms or to an inhibitory action on bone phosphatase, since both fluorine and cadmium show this property. However, while cadmium in relatively high concentration is a phosphatase poison, it has been shown by Dufait and Massart (8) that at low concentrations (10^{-3} *M* Cd ions), cadmium actually catalyzes the transmission of phosphate from phosphopyruvic acid to glucose. Fluorine in very small concentrations also has an effect of stimulation on phosphatases. However, due to the great affinity of the apatite of enamel for fluorine, the amounts taken up on exposure to this element are relatively great, and the inhibitory action on phosphatases is to be expected. The enamel has no such affinity for cadmium, and thus only takes up a very slight amount. The distribution of this may be uneven, and at some points the concentration may be sufficiently high to exert an inhibitory action on phosphatases which encounter it. In most places, the low concentration may actually stimulate acid production. In this way the variable results on progress of caries and the frequent increase in such progress may be explained.

SUMMARY

1. When 0.004% cadmium chloride is administered to rats in drinking water during the period of calcification of their molars, it is not taken up by the calcifying tooth.

2. When the drinking water contains cadmium from birth to 123 days of age, the molars acquire an average of 0.003% cadmium.

3. The presence of this amount of cadmium does not increase the number of carious lesions produced by the coarse corn diet, but it does appear to increase the rate at which caries progresses.

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DENTAL CARIES IN THE SYRIAN HAMSTER[1]

I. The Character and Distribution of Lesions

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The investigation to be reported was undertaken to determine the time of occurrence, characteristics, and distribution of carious lesions in the molar teeth of Syrian hamsters and to compare the apparent mechanisms of dental caries in this animal with that described in other species, and in man.

EXPERIMENTAL

Group A. Eighty-five hamsters (55 females and 30 males) about 35 days of age were selected from 16 litters raised on Purina Rabbit Checkers and fresh green alfalfa. All animals were marked, distributed into cages containing wood shavings, and placed on a high carbohydrate diet of whole wheat flour 20%, corn starch 25%, confectionery sugar 20%, whole powdered milk 30%, and alfalfa 5%. Supplements of fresh carrots or alfalfa were provided once a week. The prepared diet and tap water were available *ad libitum*. Twenty older animals (10 females and 10 males), taken from breeding stock, were examined for caries and periodontal disease under ether anesthesia and placed on a similar regimen.

With few exceptions, 4 male and 7 female animals were sacrificed every 10 days over an 81 day period. After fixation in 10% formalin, the jaws and teeth were examined under a low-power dissecting microscope (1.5 × 10) for stains, debris accumulations, plaque-like material, and gingival disturbances. The soft tissues were then stripped from the jaws and the teeth examined for evidence of caries activity. All findings were recorded on special charts. Numerous ground and decalcified sections were prepared, especially of those teeth which showed incipient lesions.

Group B. Because of the early appearance of carious lesions, some of the work was repeated 1 year later by placing 21 newly weaned animals on a regimen similar to that described except that no supplement of greens was provided. Six animals were sacrificed before the experiment; 7 animals, at 11 days; and 8 animals, at 20 days. Gross and microscopic examinations of the teeth were

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made. Findings confirmed earlier observations and are included with the description of the original data.

STAINS AND DEBRIS ACCUMULATIONS

Three distinct types of stain or pigmentation were found on hamster molars:

1) All animals sacrificed at the beginning of the experiment had deposits of dark green-black stain irregularly distributed over the supragingival third of buccal and lingual molar surfaces and in sulci, grooves, and fossae (*fig. 4*). This accumulation, possibly a previous dietary residue, was easily removed by gentle scraping. Although this deposit occurred in a high percentage of animals sacrificed during the first 30 days, it became less evident after 50 days and was present only in trace amounts in animals on the diet 60 days and over. It presence was not associated with caries activity.

2) A supragingival yellow-brown stain has been found frequently on buccal and lingual surfaces of hamster teeth, particularly on first molars. Although not a common finding in this experiment, this stain may have a uniform distribution or appear as concentric bands encircling cusp surfaces from the top to the gingival border. Apparently the result of pigmentation in the enamel cuticle, the stain cannot be removed by scraping but floats off with the cuticle when the tooth is immersed in dilute acid. This coloration is not associated with caries activity.

3) A penetrating yellow-brown to brownish-gray discoloration of the enamel was associated with carious lesions. In very small incipient lesions these changes were often difficult to identify positively as carious. Ground sections were helpful in the identification of such areas, but even microscopic examination did not always permit positive distinction between incipient caries pigmentation and apparently normal enamel coloration.

Debris accumulations. A white pasty material, resembling the materia alba on human teeth, was found frequently on hamster molars (*figs. 1* and *2*). This substance was widely distributed over the crown surfaces, especially in those areas which showed a high degree of caries susceptibility. None was detected on the teeth of animals sacrificed before being placed on the diet; however, it was observed after 10 days as plaque-like material adherent to enamel surfaces. Subgingival deposits of this substance were numerous and often associated with caries activity and gingival recession. Supragingival collections often appeared to be extensions of large subgingival deposits and were more common along the buccal surfaces of upper molars and the lingual surfaces of lower teeth.

On teeth of animals fed the diet 30 days and over, amounts varied from gross collections covering large surfaces of the crown to minute subgingival deposits detectable only after stripping away the gingiva. Deposits were also present in sulci and fossae. Smears of this material stained with methylene blue or crystal violet revealed amorphous material and a variety of micro-organisms most of which were spherical in form.

In 50% of animals on the diet up to 39 days, particles of fibrous material were found impacted between molars and in occlusal sulci and fossae. Maxillary

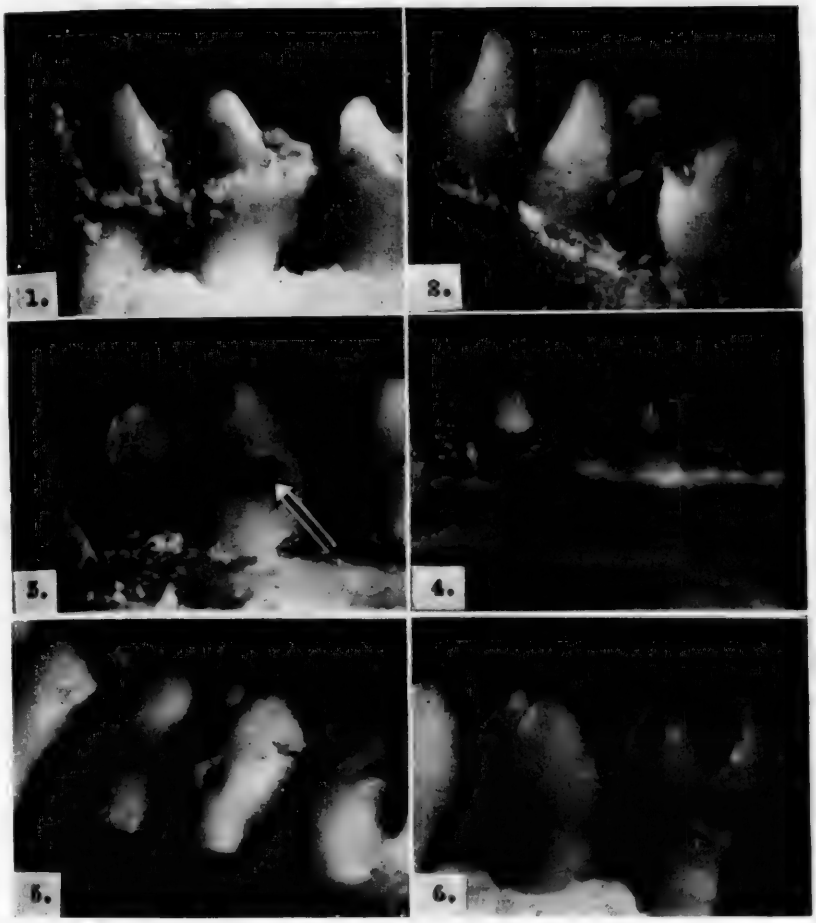

Fig. 1. Plaque accumulation of lingual surface of lower 1st molar.

Fig. 2. Localized plaque accumulation on disto-lingual surface of mandibular 2nd molar. Subgingival plaques of this type in corresponding buccal and lingual locations are associated with "interproximal lesions" See *fig. 9*.

Fig. 3. Incipient subgingival caries in enamel on lingual surface of maxillary 3rd molar. Lesion is characterized by zone of brown pigmentation surrounding small opaque area.

Fig. 4. Subgingival carious changes in enamel on buccal surfaces of maxillary 2nd and 3rd molars. Second molar has dark band of yellow-brown pigmentation with no evidence of opaque change. Third molar has similar band of pigmentation with zone of white opacity running through it. Dark black stain demarks former position of gingival border.

Fig. 5. Subgingival smooth surface lesion on maxillary 2nd molar. Lesions of this type are not uncommon but are infrequent in this particular location.

Fig. 6. Carious lesion at cervix of lingual surface of mandibular first molar. Enamel, cementum, and dentin have been affected.

impactions were much more frequent and extensive than mandibular. In animals on the diet 50 days and over, it was difficult to determine whether impactions of this material preceded or followed the loss of tooth substance due to

caries. Under the microscope this material appeared to represent small bits of wood, hairs, and cellulose fibers.

MACROSCOPIC CHARACTER AND DISTRIBUTION OF LESIONS

In Group A, the first gross evidence of caries activity was observed in 60 of 126 molar teeth (47.5%) from animals on the diet 20 days. In Group B, 1 animal showed macroscopic evidence of caries after 10 days; after 20 days, 9 gross lesions were present, all of which were less extensive than in Group A. Caries experience increased directly with the length of time on the ration as indicated by progressive enlargement in cavity size and frequency of occurrence. Maxillary teeth were affected more extensively than mandibular. Although considerable variation in caries susceptibility in animals of the same sex was observed, the caries incidence in males tended to exceed that in females. All animals sacrificed after 50 days had gross lesions and 91.6% of the teeth in animals on the diet 74 days to 81 days had carious lesions. A few females which appeared to be more resistant than others were carried on the diet for 175 days and had extensive involvement when sacrificed. No animals have been found immune to dental caries on this diet.

Older animals were also susceptible to caries activity. Pre-existing lesions were increased in extent. Periodontal disturbances were sometimes severe and carious lesions of root surfaces often caused complete loss of remaining tooth substance. Primary involvement of occlusal surfaces showing marked evidence of attrition could not be detected.

The changes in enamel and dentin were best observed after removal of soft tissues and drying the teeth. Gross evidence of incipient caries activity was more readily studied on smooth surfaces than in less accessible areas such as sulci and fossae. Incipient carious changes in enamel were of 3 types:

1) In most instances the smallest and most superficial lesions detected were but a fraction of a millimeter in diameter and appeared as circumscribed areas of brownish discoloration of the enamel surface. Neither surface dullness nor opaque changes were apparent in very early stages. Slight softness of the surface could sometimes be detected by hard scratching with a sharp instrument. In slightly more advanced lesions surface dullness and changes in enamel translucency became noticeable. Upon drying, surfaces showing these changes revealed chalky white areas surrounded by zones of brownish discoloration. These areas were readily marred with an instrument (*fig. 3*).

Opaque changes in the enamel often seemed to follow the appearance of subgingival bands of yellow-brown discoloration which encircled considerable portions of the coronal surface (*fig. 4*). If allowed to dry, enamel showing these changes generally became very friable and broke away from the underlying dentin spontaneously.

2) Another type of incipient carious change in enamel was distinguished by superficial opaque areas in which evidence of pigmentation was imperceptible or questionable. Most readily detected on smooth surfaces, lesions of this type were comparatively infrequent.

3) Enamel lesions in fossae progressed somewhat more slowly than those in broad sulci and on smooth surfaces and were associated with deeper yellow pigmentation and less evidence of opaqueness. Lesions of this type, observed in teeth of animals on other diets reported elsewhere (1, 2), have been frequently characterized by dark brown pigmentary changes, a condition which appears to be associated with lower caries activity.

Incipient lesions at the cervices of the teeth and on root surfaces were characterized by soft roughness of the cementum and early involvement of the underlying dentin. Decaying dentin was light yellow in color; however, the intensity of pigmentation in carious dentin also seems to vary with diets and with the activity of the lesion.

From evidence gathered by study of lesions of increasing size, the most frequent sequence of events in cavity development appeared to be as follows: 1) plaque accumulation, 2) superficial yellow-brown pigmentary change in the cuticle and enamel, 3) enamel penetration with opaque changes developing in zones of pigmentation, 4) enamel undermining and dentin invasion, 5) enamel fragmentation, 6) rapid and extensive cavitation.

The most frequent distribution of carious lesions may be summarized as follows:[2]

Subgingival or gingival crevice lesions (*figs. 3, 4, 5* and *6*)

Maxillary teeth

1. Almost all lesions on buccal cusp surfaces
2. Lingual cusp lesions
3. Lingual sulcus lesions on 3rd molars
4. Interproximal lesions

Mandibular teeth

1. Most lesions on buccal and lingual cusp surfaces
2. Lesions in buccal sulci of 2nd and 3rd molars may be of partial subgingival origin
3. Cervical lesions, especially on 1st molars
4. Interproximal lesions

Supragingival lesions (*figs. 7* and *8*)

Maxillary and mandibular teeth

1. Occlusal fossae lesions
2. Lesions associated with plaque accumulation or debris collections in sulci not mentioned above
3. Extensions from subgingival lesions

On the basis of frequency of involvement, certain areas of the teeth were more susceptible than others. The reason for some of these loci differences remains obscure. This is particularly true of certain subgingival areas.

[2] Methods used in this experiment did not permit a comprehensive estimate of relative frequency of each type of lesion. Such determinations would have necessitated sacrificing more than 11 animals at any interval, examination of lesions before coalescence occurred, and serial grinding of teeth from the occlusal surface to the cervical line.

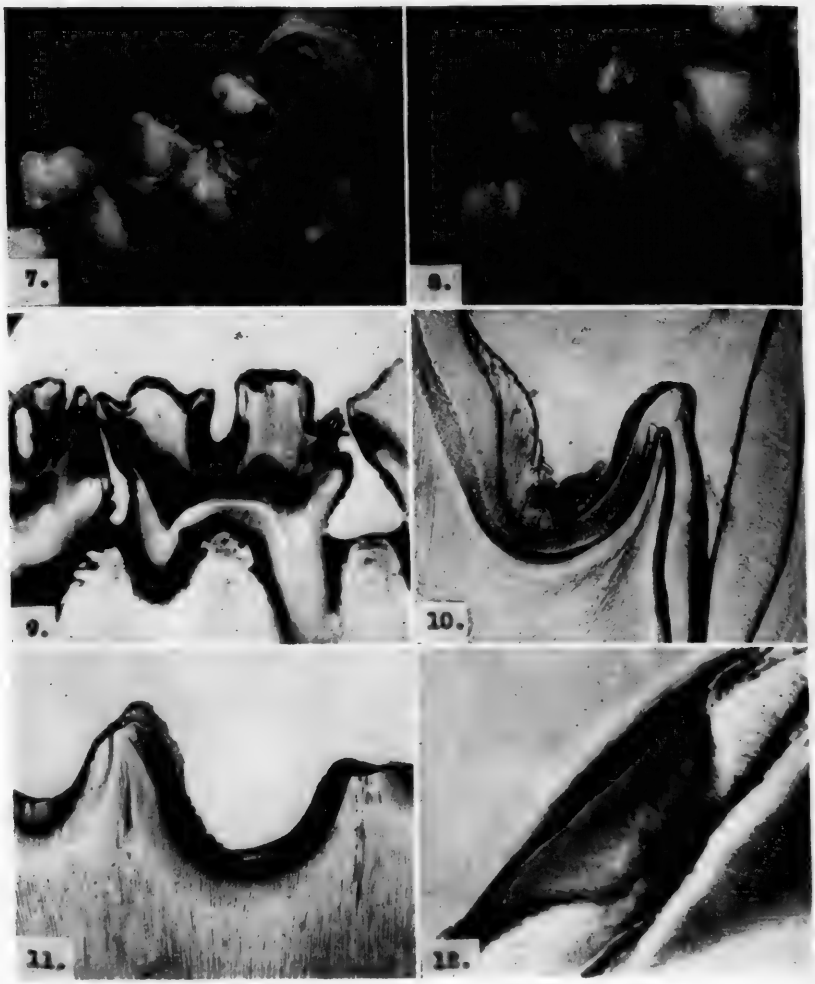

Fig. 7. Supragingival lesion in occlusal fossa of maxillary 1st molar.

Fig. 8. Carious lesion in buccal sulcus of mandibular 3rd molar.

Fig. 9. Ground section of maxillary molars stained with silver nitrate. The wide distribution of lesions is apparent. Approximating surfaces, sulci, and fossae show simultaneous involvement. Coalescence of these lesions may result in extensive cavitation. (× 30)

Fig. 10. Ground section through buccal sulcus of lower 2nd molar. Incipient carious change in enamel is associated with loose debris accumulation in sulcus, yellow-brown pigmentation, and increased distinction of interprismatic substance. (Orig. mag. × 100)

Fig. 11. Deeply pigmented carious change in enamel. Coloration deeply saturates enamel and underlying dentin. A few deeply pigmented lamellae can be distinguished in base of sulcus. Lesions of this type do not appear to be highly active. (Orig. mag. × 100)

Fig. 12. Carious lesion in enamel photographed with polarized light and red 1st order analyzer. Dark band of demarcation between carious and normal enamel is distinctly apparent. This band is more striking without analyzer but more difficult to photograph. Original magnification (Orig. mag. × 100).

MICROSCOPIC FINDINGS

Questionable evidence of caries was found in 5 teeth of Group B animals sacrificed before being placed on the ration. Of 51 erupted molars examined, in 2 maxillary second and 2 maxillary third molars, and in 1 mandibular second molar several suspicious areas were found. In both Group A and B definite microscopic evidence of caries activity was found after 10 days on the diet. In Group A, 46.1% of teeth examined in sections ground in 1 plane only showed evidence of caries after 10 days. In group B, 32.1% of 81 teeth examined showed positive evidence of caries after 10 days and 56.0% of 84 teeth showed changes after 20 days.

Ground sections. An appreciation of the wide distribution of lesions can be obtained from *fig. 9.* Sulcus and fossae lesions, smooth surface lesions, and "interproximal lesions" were a repeated finding. In ground sections through incipient lesions, several phenomena were observed which varied with source of illumination and the medium in which the specimen was mounted.

1) In unstained ground sections mounted in water or alcohol and viewed with reflected light, incipient enamel changes were associated with adherent plaque material on the surface and were characterized by changes in enamel translucency. Zones of milky opaqueness were in marked contrast to the blue opalescence of the surrounding normal enamel. Clearing and mounting destroyed this property, and lesions then appeared as slightly yellow transparent areas surrounded by blue opalescent enamel.

2) Areas which showed milky opaque changes by reflected light were always characterized by brownish pigmentation when studied by transmitted light. Carious enamel was saturated with this color and the interprismatic substance appeared more distinct (*fig. 10*). Enamel showing these changes had an affinity for silver nitrate and Gram's stain (*fig. 9*). Beneath carious enamel, affected dentin showed similar but somewhat lighter pigmentary changes. As the carious process extended into the dentin, the tubular structure of the dentin was lost, and the enamel became more friable and increasingly difficult to retain in the grinding process.

Some carious lesions have been characterized by deep pigmentary changes in enamel and underlying dentin and by less evidence of opaque change. Enamel lamellae and interprismatic substance have generally shown deep pigmentation and the process appeared to be spreading along these structures into the dentin. Carious lesions of this type have been observed more often in fossae and sulci and seem to be slowly progressive (*fig. 11*).

Water or alcohol mounted preparations of active lesions revealed zones of brilliant yellow surrounded by clear blue normal enamel when examined with crossed prisms in a petrographic microscope. Clearing and mounting changed this effect somewhat, but a distinction between carious and non-carious enamel was generally not difficult to make in well defined lesions. A dark band of demarcation between the carious zone and normal enamel could be detected. Interesting color effects could be obtained with various analyzers (*fig. 12*).

Decalcified preparations. Subgingival lesions in sections which preserved the

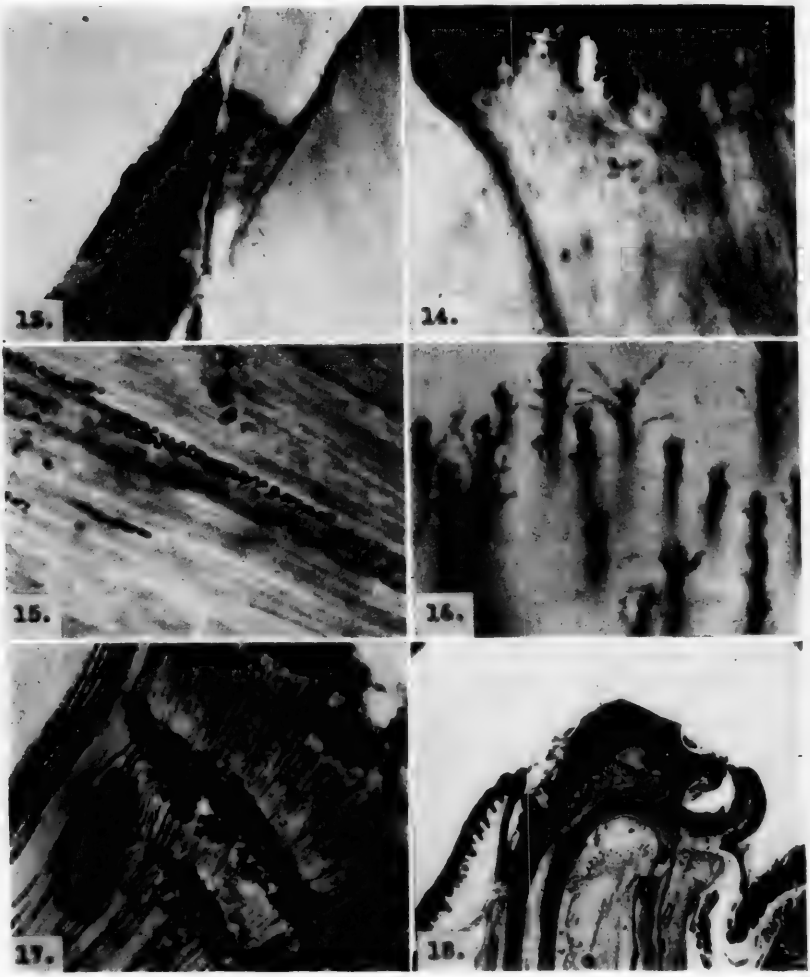

Fig. 13. Subgingival carious lesion with massive invasion of enamel. Beneath the keratin border of the gingiva, plaque material remains adherent to the enamel surface. Fine granular material is present in enamel matrix and is distributed along amelo-dentinal junction. (orig. mag. × 210)

Fig. 14. Gram-positive spherical micro-organisms in enamel matrix of hamster molar. Organisms have not penetrated beyond lamella on left, but a few appear to have penetrated along this structure. (Orig. mag. × 1500)

Fig. 15. Early tubular invasion of dentin by spherical micro-organisms. The distribution of organisms is along the wall of the tubule. (Org. mag. × 1500)

Fig. 16. Advanced tubular involvement of dentin. Gram-positive spherical forms completely fill tubules and lateral branches. (Orig. mag. × 1500)

Fig. 17. Extensive decay of dentin. Dense infiltration of tubules and areas of complete necrosis are apparent. Gram's stain. (Orig. mag. × 430)

Fig. 18. Root caries in older animal with severe attrition. Pocket formation is apparent. Secondary dentin has been deposited on pulpal walls. (Orig. mag. × 50)

cuticle and organic matrix of the enamel offered a favorable opportunity to observe phenomena associated with caries activity.

In areas showing a separation of the gingiva from the crown surface, plaque material was observed in the crevice between the keratin border of the gingiva and the enamel cuticle. Enamel beneath the plaque material contained darkly staining finely granular material. This substance was distributed along the amelo-dentinal junction and extended into the dentinal tubules (*fig. 13*). In sections stained with Gram's stain the plaque material and invaded areas were filled with masses of small round Gram-positive particles resembling spherical micro-organisms. Although other bacterial forms were found in and around plaques, spherical types predominated. Spherical forms have been observed in the enamel matrix and in advancing lesions in dentin (*fig. 14*). In dentin the spread of micro-organisms was far in advance of areas showing complete necrosis. The distribution of organisms in the tubules varied with their distance from the surface of the lesion. Following enamel penetration, micro-organisms seemed to spread along the amelo-dentinal junction and then invade deeper areas of the dentin. In early stages of dentin invasion, the micro-organisms were distributed around the periphery or wall of the tubule (*fig. 15*). More extensively involved tubules were completely filled with organisms, and eventually appeared to increase in diameter and lose their integrity (*figs. 16* and *17*). Small aggregations of micro-organisms were found collected in foci in which the dentin showed complete loss of structural detail. Pulpal invasion was observed in advanced lesions.

DISCUSSION

The purpose of this experiment was to ascertain the early character and distribution of carious lesions in hamster molars, to follow the development of these lesions, and to determine general similarities and differences between caries activity in hamsters and in other species.

On the diet used for this study Syrian hamsters were susceptible to a highly active form of dental caries. The finding of microscopic carious lesions in teeth of animals on the diet 10 days and gross lesions after 20 days is interesting and in contrast to most reports on rat caries. This rapid development of lesions compares favorably with the early appearance of lesions in white rats described by Shibata (3). The observation in hamsters appears even more significant when one considers that Shibata's study was probably complicated by fractures of the teeth due to coarse rice used in his rations and that his finding of lesions in 20 days has not been generally confirmed (4, 5, 6).

That animals should be placed on experimental diets as soon as possible after weaning is suggested by the finding of questionable lesions in several teeth of Group B animals sacrificed before being placed on the ration. Animals in Group A had more gross lesions at the end of 20 days than Group B. This may be a chance variation or due to the fact that Group A animals were about 10 days older than Group B animals when started.

Both supragingival and subgingival lesions were related to the presence of

adherent plaque material on the surfaces of the teeth. Evidence of detachment of the enamel cuticle from the gingival epithelium was found, and this condition seemed to predispose the animals to subgingival and crevice caries. The fibrous material found in the sulci and fossae of the teeth was not a tight impaction but more of a loose collection which in no way was associated with fractures of the teeth. In areas where this material collected, it may have been conducive to plaque accumulation and thus predisposed certain areas to caries activity. However, such material was not always present, and many lesions were observed without any evidence of it. Fibrous matter was more common in the gingival tissues of the maxilla than in those of the mandible. The impaction of fibers in the gingiva may have contributed to the gingival detachment and subjected the animals to periodontal disturbances and subgingival caries (*figs. 19* and *20*).

Interproximal lesions in hamsters are not considered comparable to the same lesion in humans. Although such lesions are interproximal in location, appar-

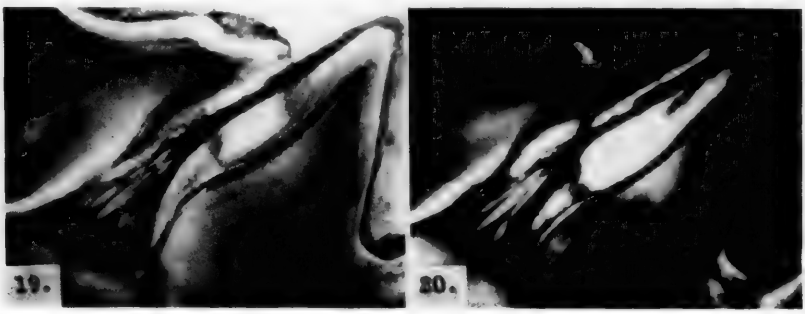

Fig. 19. Contact surfaces of maxillary 2nd and 3rd molars showing carious lesion in enamel and impaction of hairs in gingival tissues. Photographed with polarized light and red 1st order analyzer. Original mag. × 100.

Fig. 20. Same area as *fig. 19* under polarized light without analyzer. Three hairs in gingival tissue are more distinct.

ently they do not originate on approximating surfaces or in the interproximal spaces. From subgingival plaque accumulations on adjacent tooth surfaces both buccal and lingual to contact areas, the carious process penetrates the teeth and spreads between them, so that in the plane of sagittal sections lesions appears to be interproximal. Actually these are smooth surface lesions which originate beneath the buccal and lingual papillae and spread to contacting areas (*fig. 2*).

Since the carious process generally attacks the teeth in several areas simultaneously with rapid coalescence of lesions and extensive cavitation, it has never been considered advantageous to specifically localize lesions by arbitrary areas. Although such methods have proved helpful in recording rat caries (7, 8), the applicability of such procedures to hamster caries has not seemed practical.

Although many pathological processes and associated phenomena appear quite similar in both hamster and human caries, related mechanisms are often

distinctly different. Subgingival lesions are not of frequent occurrence in human beings. Root caries may occur in humans with periodontal disease and physiologic root exposure, but this is not a common finding. Interproximal lesions in hamsters and coronal lesions associated with fiber impaction are not comparable to lesions in humans. Caries activity, as it occurs in occlusal fossae and some sulci of hamster molars, appears to be analogous to pit and fissure caries in man, when not complicated by fiber collections. Lesions in certain buccal and lingual sulci are occasionally subgingival in origin and, therefore, are not always comparable to human lesions. Cervical lesions in hamsters are preceded by gingival recession and begin either in cementum, cervical enamel, or both. Teeth severely worn by attrition may have extensive lesions on root surfaces. Caries activity in these areas generally follows periodontal disturbances. (*fig. 18*).

Coronal lesions in hamsters were associated with plaquelike material and showed enamel penetration and dentin involvement before loss of hard tissue. Pigmentary changes in enamel and dentin were similar in many respects to those described in human teeth (9). In ground sections, zones of enamel-dentin separation were sometimes observed before loss of surface continuity of enamel. Frisbie (11) ascribes areas of "dentin shrinkage" to drying of the specimen in the process of preparation. The possibility of this occurrence cannot be excluded in some of the sections. Although a study of decalcified tissues suggests that a potential space may exist between carious enamel and dentin, in sections prepared with particular precaution to prevent drying, this space has been filled with fine granular material.

Modifications in histologic technic and the extreme minuteness of the structural components of hamster enamel and dentin have not permitted a study of the finer histopathological details described by Frisbie, Nuckolls, and Saunders (10) in human caries. However, sufficient data have been obtained to establish a remarkable resemblance between the fundamental aspects of the carious process in hamsters and humans.

In dentin the tubular distribution of micro-organisms seems to correspond to that described by Frisbie (11): "Initial tubular penetration and continued ingress of micro-organisms is along the calcifying matrix bordering tubules." "In more extensive superficial positions organisms are found within dentinal fibril and lateral branches." Aggregations of micro-organisms in localized areas of dentin necrosis resemble Frisbie's description for human teeth, "With involvement of many lateral tubules and accompanying extension of the proteolytic process coalescence occurs forming localized irregularly shaped areas of disintegration of dentinal structure."

The demonstration of spherical Gram-positive micro-organisms in carious lesions is interesting in view of numerous attempts to induce caries activity in rats by inoculation of the oral cavity and diets with strains of *Lactobacillus acidophilus* and other organisms associated with human caries (12, 13, 14, 15, 16). However, coccoid forms have been associated with dental caries in rats. In cultures from carious rat molars, Harrison (17) reported 60% of organisms were

acidogenic streptococci and lactobacilli were found in small numbers. He later reported (18): "In 8 out of 10 carious teeth streptococci made up over 90% of the total acidogenic flora". "Lactobacilli were cultured in small numbers from three teeth on which enamel caries was found . . . ." He suggested that lactobacilli were associated with caries activity at the tooth surface and acidogenic streptococci with advanced decay.

The demarcation bands and other changes between normal and carious enamel under polarized light are not unlike those described by Applebaum in human teeth (19): "By polarized light . . . different layers or zones are more definitely shown by various color effects." "The most striking zone is the band of demarcation separating the outer cone of demineralized or carious enamel from the unaffected". No attempt is made to interpret the phenomena displayed in hamster enamel by polarized light other than to suggest that they probably represent changes in the crystalline pattern of the enamel concomitant with caries activity. It should be mentioned that study of enamel by polarized light has not proved helpful in the diagnosis of incipient lesions. In areas which show unmistakable evidence of caries activity, polarized light reveals striking changes. However, in other sections somewhat similar effects have been observed in apparently normal enamel. The reason for these results are not known but may be due to variations in grinding the sections, to variations in the course of the enamel rods, to differences in the crystalline arrangement in different zones, etc.

Technical difficulties in handling and observing hamster teeth make it unlikely that these animals will be suitable for studying the minute histopathological details of caries activity. However, conclusive evidence has been obtained to demonstrate the true carious nature of the lesions and the fundamental similarity to carious processes found in higher animals.

Additional information on mechanisms associated with caries activity in this species is needed. Investigations on the role of fibrous material are now in progress. The facts that this animal is readily available, easily raised and handled in the laboratory, has a molar dentition more comparable to the human than that of the rat, and that true dental caries can be produced on a wide variety of rations without excessive sugars and cusp fractures deserve consideration by those interested in experimental caries studies.

SUMMARY AND CONCLUSIONS

One hundred and twenty-six hamsters were placed on a high carbohydrate diet of whole wheat flour, corn starch, sugar, powdered milk, and alfalfa. Carious lesions were detectable microscopically in the teeth of animals on the diet 10 days. Gross lesions were frequent and extensive after 30 days.

Carious lesions in the molar teeth were associated 1) with the accumulation of plaque-like material, 2) with yellow-brown pigmentation which preceded gross loss of calcified tissue, 3) with striking changes in enamel translucency, 4) with the presence of Gram-positive spherical micro-organisms in both enamel and dentin.

Because of marked species differences, all comparisons of dental caries in hamsters with that in humans must be made with reservation. However, the conclusion has been reached that the fundamental process of dental caries in hamsters appears very similar to that described in humans. Such differences as there are seem to be more of a quantitative than qualitative nature.

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SERIAL SECTIONS OF UNDECALCIFIED ENAMEL[1]

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For a thorough microscopic examination of some animal tissue it is necessary to cut microscopic sections of such tissue, i.e. slices of from 5 to 100μ in thickness, which can be studied under the microscope in transmitted light and by means of high power lenses. For this purpose we possess microtomes which will cut slices of any thickness. Most animal tissues lack the consistency which allows of extremely thin slices being cut, they have to be frozen or to be embedded in paraffin or celloidin.

The study of a single section is not always sufficient to enable us to answer all questions. It may be that the object of our study extends over layers of tissue above or below the cut. Then the system of making an uninterrupted series of cuts through the tissue serves to render every part of it accessible. No detail, however small, can then escape our microscopical inspection, and objects which extend over many sections can even be reconstructed.

To a certain extent the histological study of enamel has been on the above lines. The stage of single sections was soon reached, although the technic was quite different, for the tooth or the piece of enamel was simply ground down until a slice thin enough for microscopic examination remained. In order to obtain serial sections of the enamel there are only 2 alternatives. One group of investigators used the microtome on enamel embedded in some suitable medium. Bödecker (05, 08) attained excellent results with his celloidin method, in which decalcification and embedding take place in one process, but in these sections he investigated enamel deprived of an essential ingredient, viz., the calcium salts. In his investigation, the special aim of which was to study the organic constituents of enamel, this was an advantage, but in the majority of cases it renders the method wholly unsuitable. The other method is as follows: by means of a diamond disk one saws from the crown of a tooth as many slices as possible and grinds them down to the required thickness. It is evident that in this method serial sections in the true sense of the word are out of the question for in the sawing and grinding processes much material is lost as sawdust.

In order to limit this loss we constructed a sawing microtome adapted for cutting thin serial sections. For cutting thin slices of bone Arndt (61) used a kind of strong fret-saw in which 2 fine parallel saws were fitted close together. For enamel he judged steel saws too soft and he suggested the use of 2 diamond disks in a similar way.

[1] Received for publication June 20, 1946.

Most later authors (Black '15, Bödecker '21, '26, Patten '25) who applied sawing-methods in order to obtain serial sections of enamel made use of the fact that soft metals retain bits of grinding-powder (emery, carborundum, etc.) on or rather in their surface, for by pressing a rotating aluminium circular saw tightly against a block of some hard material (e.g. glass) and applying an adequate quantity of emery powder suspended in water, it appears to be possible to make a cut in the glass, although the latter is much harder than aluminium. On scrutinizing the aluminium disk it appears to have been roughened at its periphery and covered with numerous grains of emery, stuck fast during contact between disk and glass in the soft material of the disk, their protruding edges acting as the teeth of a saw. The grains of emery are not firmly fixed in the material of the disk and probably they are loosened during the next passage along the glass, and perhaps take a few particles of the aluminium along with them. From this it follows that if a continuous supply of emery is not provided the disk will soon be blunt and will gradually wear off at its periphery. The above mentioned authors made use of this property of aluminium disks to saw enamel into slices. To this end the rotating aluminium disk was fixed in a lathe in such a way as to make it possible to move the tooth towards the disk along a robust slide-bar and then to shift it over a previously determined distance for the next cut to be made. The grinding-powder suspended in water was evenly distributed over the surface of the disk by means of felt disks. Similarly, Lefkowitz (40) used a "diamond-charged phosphobronze saw", by means of which he evaded the difficult problem of a uniform supply of emery. It remains to be seen, however, whether the diamond disk may be made as thin as the aluminium one.

What is achieved with this method? To answer this question one has to bear in mind that all authors apply the sawing-method only as the initial stage in the production of their slices, which are afterwards ground to the proper thinness. Bödecker is the only one who stated his results clearly: he managed to produce 1 section per 2 mm. of tooth. Lefkowitz stated that his sawed slices are 250μ thick, but he failed to inform us as to the width of the sawcuts between the slices.

These results are not encouraging. For the microscopic examination of small foci of caries incipiens, e.g., it is desirable to have at least 2 or 3 sections per millimeter at our disposal; it is not necessary for these slices to be sections through the whole element. We have therefore asked ourselves whether it would be feasible to cut more than 1 slice per mm. of at least part of an element, e.g., of the enamel and of the adjoining dentin of the approximal side of a premolar. Now, the distance between 2 slices is determined by the breadth of the sawcut and the quantity of the material lost while the slices are being ground to the required thinness. The sawing is essential, the grinding is only necessary if the sawing-method produces too thick slices. So it was desirable to evolve a sawing-process which in the first place makes very narrow sawcuts and, secondly, produces slices so thin that subsequent grinding is superfluous (sections of from 50 to 100 μ in thickness are not too thick for most investigations).

We have chosen a method in which the periphery of a disk composed of some soft material is coated with grains of grinding-powder. The breadth of the sawcut is determined by the thickness of the disk, the amount of wobbling of the disk, the play in the mechanism, and the thickness of the layer of grains stuck fast in the sides of the disk. The thickness of the metal disk cannot be reduced too much, for a disk of some breadth, which is required to produce a sawcut of some depth, would become too flexile and might easily deflect. The active breadth of the disk is less than its radius, as for various reasons it has proved practical to mount the sawing disk between 2 smaller and thicker disks, so that the ring protruding from the two thicker disks is actually used in the sawing process (*fg. 4a*). In the case of an active breadth of 5 mm. a disk 18 mm. thick of a special soft sort of aluminium is still sufficiently rigid. It is evident that in the case of a wobbling disk the "effective thickness" is greater than the actual thickness. Fixing and directing the disk is therefore an important factor in our endeavour to achieve a narrow sawcut. Wobbling is harder to eliminate in a large disk than in a small one. But a large radius is desirable, for a large disk does not wear down so rapidly and with a given active breadth is capable of making longer sawcuts than a small one.

Similar to the consequences of a wobbling disk are those brought about by insufficient stability in the bearing of the disk-shaft and in the mechanism which feeds the tooth to the circular saw as the sawcut deepens. The layer of grinding powder between the sides of the disk and the material to be sawn is kept thin by choosing a very fine powder and giving a scanty supply, so that between the material and the disk there are no more grains than are required to replace those that have been loosened. That the factors mentioned (and perhaps others still unknown) are not negligible as compared with the influence of the actual thickness of the disk, appears from the fact that we have not succeeded in reducing the sawcuts to less than 300μ although the disk was only 180μ thick.

Without special precautions it is impossible to saw slices of 100μ in thickness without the risk of the slices crumbling off during the sawing-process or of cracks forming in them. Evidently slices of so small a thickness cannot withstand the lateral pressure exerted on them by the disk. For this pressure 2 factors are responsible. The first is the lateral pressure at the bottom of the sawcut. The fact is that during the sawing process the cutting edge of the disk becomes convex in profile and therefore not only exerts pressure in the direction of the sawing but also laterally. As this lateral pressure however works at the root of the slice, little detrimental effect is to be expected. This cannot be said of the second factor, arising from the presence of grinding powder in suspension between the side of the disk and the surface of the slice, for this force operates much farther from the root of the slice. It is especially this second factor which we must try to eliminate. This may be achieved by thinning the aluminium disk out towards its centre. Drawbacks of this construction are that such a disk, when of equal thickness at its periphery, is weaker than one of uniform thickness, and that the thickness of such a disk continuously decreases at its periphery as it wears down. Another possibility is to limit the supply of emery in suspension to the periphery

of the circular saw. Finally one may try to protect the slice against lateral pressure by supporting it during the sawing process.

Some other points have to be discussed in brief. When a tooth in an object holder is pressed against the circular saw with a predetermined force, one cannot be sure that a uniform pressure willl be maintained. This requires some explanation. Even if one starts with a saw perfectly circular and centered, its original shape will get lost owing to wear and tear (lack of homogeneousness of the material and inequality in thickness of the various parts of the disk). If, even under these circumstances, the whole periphery of the disk is to be utilized the tooth must be pressed against the disk through the medium of a spring as a guarantee that the contact between the saw and the tooth will be maintained throughout each revolution. So long as the disk revolves slowly the tooth can easily make the necessary movements relative to the disk, but if the latter rotates very rapidly—which is necessary to make it possible for the work to proceed with reasonable speed—these movements have to be so fast that the inertia of the object holder and tooth comes into play, so that the forces acting between the tooth and the saw increase and decrease alternatively far more than one expects from the elasticity of the spring. Hence the mass (for inertia is a function of mass) of the object and object holder should be reduced as much as possible. By this means the action of too strong forces can be prevented and, with that, consequent damage to the object, rapid wear and even deformation of the disk.

If we try to continue sawing until the whole slice has been cut, it will break off and be flung away by the rapidly rotating disk. It is therefore convenient to embed the object and continue the sawcuts beyond the tooth into the embedding mass; by means of a thin spatula the slices thus formed can then be broken off along their inner edges.

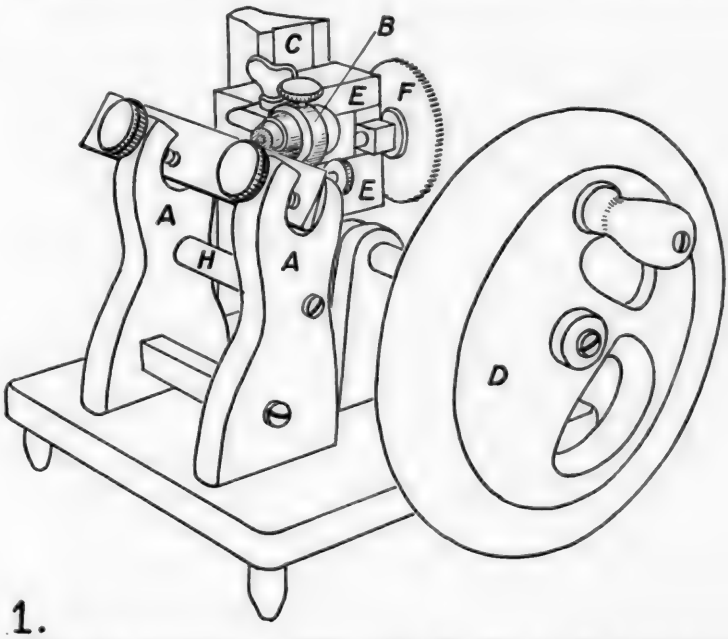
DESCRIPTION OF METHOD

1. *The apparatus.* For the construction of the apparatus a Minot microtome (*fig. 1*) was used. A is the knife holder. The object holder B is fixed to the "slide-rest", which can be raised or lowered along the prismatic column C by turning the wheel D, the rotation of which is transmitted to the slide-rest by a crank. B can be shoved horizontally towards the knife along the guides E by means of the micrometer-gauge F. As the object holder descends a slice is cut off the object by the knife, which itself does not move. Before one can proceed to cut the next slice the object has to be brought above the level of the knife again (by turning D) and shoved towards the knife over a distance equal the thickness of the slice to be cut (by turning F).

The following alterations were made in this microtome. The knife was replaced by a circular saw. To this end a tube G (*fig. 2*), containing the bearings of the shaft on which this saw revolves, was firmly welded to one of the transverse bars (H) of the (now inverted) knife holder A. *Fig. 3* is a vertical section through the circular saw, bearings and driving gear. The saw, is firmly held in position between a disk fixed to the shaft and a loose brass disk. A nut presses the loose disk against the saw, which in its turn is pressed against the fixed disk. The

shaft is borne by the ballbearings, which do not allow of any lateral play. Beyond one set of bearings (6) the shaft thickens towards the disk, so that shifting of the shaft in bearing 6 in the direction of 7 is out of the question; shifting in opposite direction is prevented by ring 8 and nut 9. Loosening of the latter is rendered impossible by the check nut. Between the 2 nuts the pulley is placed; the driving cord passes over it. The outer rings of the bearings 6 and 7 have been forced into the ends of tube G (see also *fig. 2*), welded to the transverse bar H of the knife holder. The dimensions of the disk are expressed in mm.

It was an obvious thing to use the mechanism of the microtome for the movements of the object in relation to the saw-disk, consequently to fix the tooth in

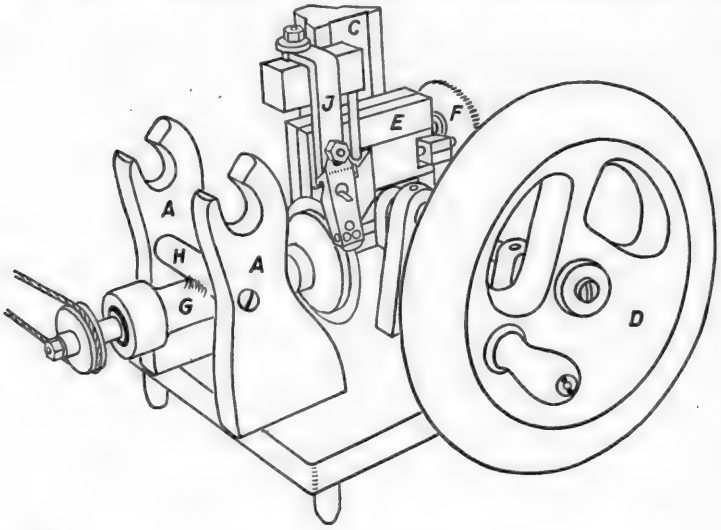


1.

Fig. 1. Minot type microtome. A—Knife holder, B—slide rest, C—prismatic column, D—wheel, E—guides, F—micrometer gauge.

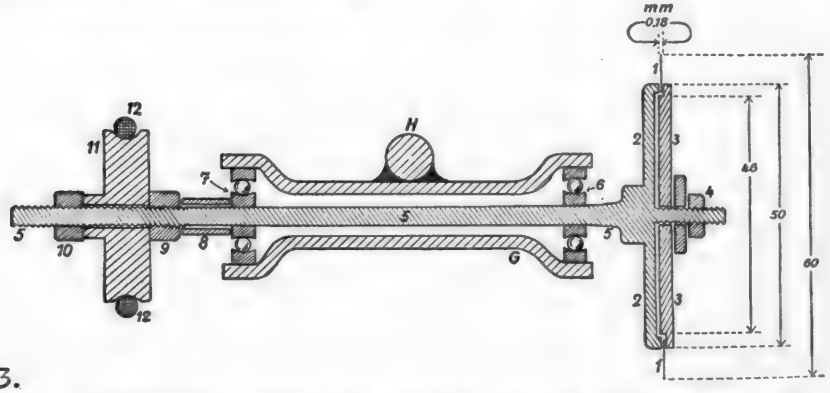
the object holder B (*fig. 1*), to lower it towards the saw by means of the wheel D, with a view to making a sawcut, and then to shift it laterally for the next cut. It appeared, however, that it is impossible to regulate the upward and downward movements with sufficient accuracy, even if an hydraulic braking-mechanism is applied. (The mass of the tooth and object holder is, indeed, very considerable in this case and we remarked above that this is detrimental to the sawing-process.) Therefore a special mechanism was constructed, which made it possible to reduce the mass of the object and object holder very considerably. This mechanism—which takes the place of the object holder B in *fig. 1*—is drawn on a larger scale in *figs. 4a, b and c.* J(see also *fig. 2*) is a vertical brass bar,

bearing at its base an horizontal steel spindle, exactly parallel to the shaft of the saw-disk. Number 14 is the object holder proper, an aluminium plate with the



2

Fig. 2. Modified microtome for undecalcified sections. A—Knife holder, C—prismatic column, D—wheel, E—guides, F—micrometer gauge, G—tube containing bearings of shaft, H—transverse bar, J—vertical brass bar.



3.

Fig. 3. Vertical section through saw, bearings and driving gear. 1—Saw, 2—disc fixed to shaft, 3—loose brass disc, 4—nut, 5—shaft, 6 and 7—ball bearings, 8—ring, 9—nut, 10—check nut, 11—pulley, 12—driving cord.

edges turned up. Both these edges are provided with notches (15 and 16) near their tops in such a way as to make it possible for the object holder to rest on the spindle and turn on it. This rotary motion cannot exceed some 10 degrees,

however, for it is checked either when the wings 17 of the object holder touch the bar J or when they come up against the nut 18 on the screw 19. To bar J (*fig. 4c*) a spring is attached, whose loose end is passed through an opening in the object holder. This spring presses the notches of the object holder on the spindle and turns it towards the circular saw, but at the same time shifts it along the

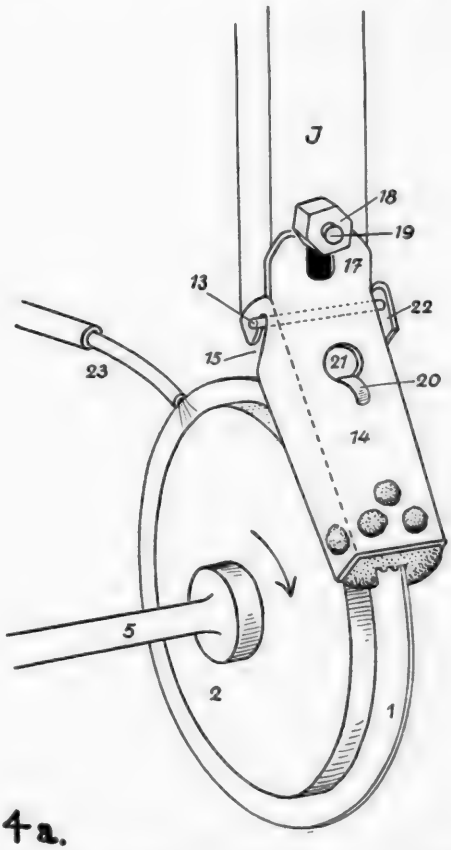


Fig. 4a. Details of mechanism replacing object holder. J—vertical bar, 1—saw, 2—disc, 5—shaft, 13—spindle, 14—object holder proper, 15—notch, 17—wings of object holder, 18—nut, 19—screw, 20—spring, 21—opening, 22—doubled edge, 23—tube (*ca*, 1/2 size).

spindle until the doubled down edge 22 over the notch is stopped by one end of spindle 13.

The object (part of an element) is fixed in the lower part of the object holder with the aid of modeling cement. The holes in this part of the object holder provide the cement with a firmer hold.

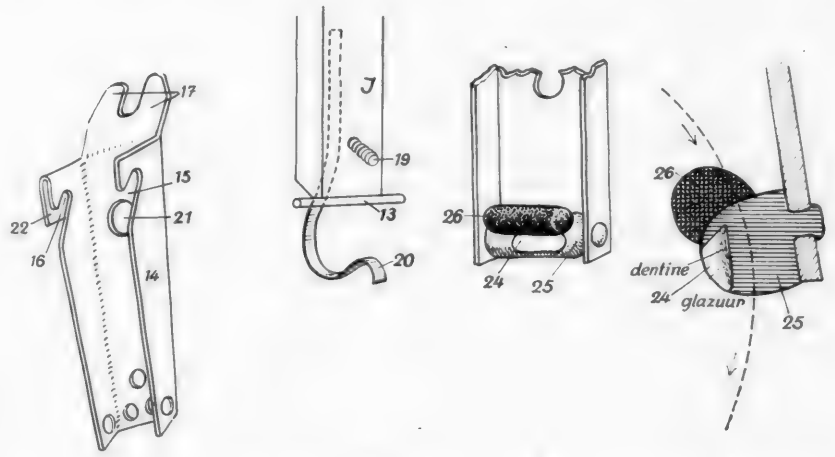
The object holder is turned away as far as possible from the disk before the

sawing starts (the wings 17 rest against the bar J). The element approaches more and more the centre of the saw disc while the sawing is in progress by rotation of the object holder around the spindle. This movement is stopped the moment the wings touch nut 18. Consequently the position of this nut determines the depth of the sawcut.

The construction of this mechanism had to fulfill 2 requirements: first, the object holder had to be very light in weight, and secondly, its removal and replacement in exactly the same position had to be a simple matter, for it is desirable to remove each slice before sawing the next one, and it is much easier to break a slice off along the embedding mass and catch it as it falls down, if to this end the object together with the object holder is taken out of the apparatus altogether for a while. This can be easily done by unhooking the object holder from the spindle. On replacing it great care should be taken to make the doubled edge 22 touch the end of the spindle in order to prevent the object holder moving along the latter holder. This is absolutely essential, as the adjustments for the successive sawcuts by means of the micrometer gauge go in the same direction, so that any unintentional shifting along the spindle would increase or decrease the distance between 2 sawcuts to an extent which could not be checked.

During the sawing a regular supply of water and grinding-powder must be maintained, but it is not necessary to supply the powder suspended in water. In the apparatus under discussion water is supplied to the periphery of the disk by a tube (23, *fig. 4a*). It spatters from the rapidly revolving disk in every direction and must be caught by an accurately fitting baffle plate. To this end the saw disk is mounted in a tin box, (not represented in the picture), having an opening in the lid only. All the splashing water is collected at the bottom of this box and drawn off by a water-jet air-pump. While the mechanism operates the spindle 13 is just below the level of the lid of the box. The slide rest of the microtome is then in its lowest position and the object holder (14 in *fig. 4a*) is in the box in its proper position with regard to the saw-disk. When by turning the wheel D the slide rest is raised, the bar J emerges from the box together with the object holder. In this position of the slide rest the object holder can easily be hooked and refixed. Practice has taught that it is desirable to supply the grinding powder sparingly and to limit the supply to the periphery of the disk. With a view to this the method we applied is such that the saw not only cuts through the tooth and the embedding mass but also through a small block composed of grinding powder and a small quantity of cement formed into a compact mass, which should not be too hard. It is practical to embed this block together with the object in such a manner that the saw first cuts through the block and then through the object, so that the cut progresses simultaneously in both. It was expected—and the result confirmed our expectation—that during its passage through the block of grinding-powder the disk would take along with it a sufficient amount of powder for the sawing process. Besides—if the block is of the right consistency, so that the grains are loosened by the pressure of the disk only and not washed out by the water—all the necessary grinding powder is taken exclusively by the edge of the disk, so that the supply of grinding powder is not only scanty but also strictly limited to the edge of the disk.

Fig. 5 shows the side of the lower part of the object holder 14 (*fig. 4*), turned towards the saw-disk. Number 24 is a fragment of a tooth, 25 a block of modeling cement, in which it is embedded. If the cement is applied in a plastic condition, it bulges through the holes in the object holder, so that after hardening the block is firmly fixed. Number 26 is the mixture of grinding powder and cement, which is likewise applied in a plastic state and is so modeled that in any case the saw-disk has to pass through a certain thickness of it before sawing into the tooth and the embedding medium. In *fig. 4a* a few sawcuts have already been made and the sawn slices broken off. It is important that the fragment of the tooth should have about the shape sketched in profile in *fig. 6*, drawn parallel



4c 4b 5 6

Figs. 4b and *4c*. Further details of modified object holder. J—vertical bar, 13—spindle, 14—object holder, 15 and 16—notches, 17—wings, 19—screw, 20—spring, 21—opening, 22—doubled edge.

Fig. 5. Lower portion of object holder. 24—tooth fragment, 25—modeling cement, 26—mixture of grinding powder and cement.

Fig. 6. Object holder detail parallel to sawing plane. 24—tooth fragment, 25—modeling cement, 26—cement and grinding powder mixture, glazuur—enamel.

to the sawing plane (the direction in which the disk turns is indicated by arrows), for otherwise sharp edges may appear on the enamel during the sawing, which will scrape all the grains of grinding-powder off the disk. If such a thing happens the disk will wear very rapidly and become incapable of sawing enamel.

APPLICATION OF TECHNIC

After the detailed description given of the various parts of the apparatus, its use can be described in few words. An electric motor at about 1000 r.p.m. drives the saw disk. The cutting and grinding of the fragments of the tooth are done by means of a diamond disk and pieces of carborundum. For reconstruction purposes it is important to have planes of reference of known orientation. For embedding the fragment of the tooth we used "Petralit" modeling cement,

as grinding stuff a mixture of this cement with a high grade emergy-powder (at the time of our experiments high grade homogeneous kinds as Alundum E 105 were not available in Holland) in the ratio 1:6 or 8, with as much moisture as is necessary to obtain a plastic mass. In 20 minutes this paste hardens to the proper degree of hardness; if one waits too long or takes too much cement the block may become so hard that the disk cannot scrape off any grinding-powder and polishes itself as if against a grindstone.

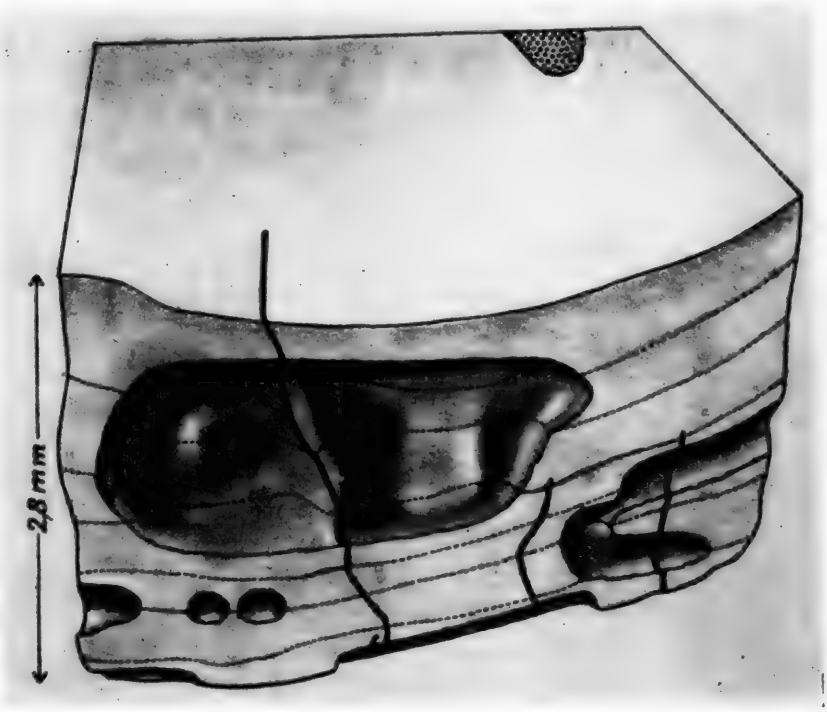


Fig. 7. Reconstruction of area of caries incipiens

After sawing a slice (which takes 3 or 4 minutes for a sawcut of 5 mm. in depth), the slide rest is raised by turning the wheel D, the object holder is unhooked, the sawn slice removed in the way described earlier in page 358, and the object holder put back in exactly the same position as before. Then by means of the micrometer gauge the object is moved over the proper distance, the slide rest is lowered again, so as to bring the object holder in its proper position facing the disk, and the next sawcut is started.

The separate slices are first immersed in absolute alcohol, and then via benzene brought between object and cover glass in cedar oil or balsam. They are then ready for microscopic examination.

In this way we can prepare series of 10 or 12 slices of parts of elements. These

slices are 3 mm. broad, 4 or 5 mm. long, and 50-100μ thick. Their thickness and smoothness of surface are such that they are suitable for microscopic examination even under high power lenses and in polarized light; for most investigations it is quite unnecessary to grind them thinner or smoother. Between the slices from 300 to 350μ of enamel is lost, so that we get one slice out of every 400μ that is 5 slices per 2 mm. This shows that, although it is certainly not the last word in serial slices, our method is a considerable improvement on previous existing methods for the examination of undecalcified enamel.

As an illustration of what can be achieved with this method is given a picture of a graphic reconstruction based on a series of sections through a region of caries incipiens (*fig. 7*) on the proximal side of a premolar. The element was cut after lying for some weeks in a solution of diamond fuchsin, a deep-red dye. Decalcified and carious spots are dyed red in it, the dyestuff also penetrating into the lamellae. In the drawing of the slices not only the outline of the enamel and the course of the lamellae but also the lines of demarcation of the red and the uncolored enamel were exactly marked. The reconstruction, made with the help of the drawings thus obtained, only shows sound uncolored enamel. Therefore the decalcified colored spots in the enamel have been reproduced here as cavities. The depth and the relief of the bottom of these cavities indicate how far the coloring and consequently the decalcification (if it may be presumed that decalcification leads to coloring) has proceeded into the sound enamel. The heavy black lines show the course of some lamellae. It is apparent that in this case the decalcification has certainly not proceeded farthest along the lamellae. In the large caries-spot (the word "large" is only used by comparison here, for the dimensions are about 1.5 x 2.5 mm.) the lamella coincides with a ridge instead of with a hollow, as would be the case, if the decalcification in the neighbourhood of the lamella had proceeded farthest. The thin dotted lines indicate the planes of the sections from which this drawing was reconstructed. The dotted part in the upper surface is a section through dentin.

SUMMARY

A sawing-method to obtain serial sections of undecalcified enamel is described. It has been achieved to produce series of more than 10 slices in sequence from 50 to 100μ thick out of 4 mm. of enamel. As an illustration of what has been attained a graphic reconstruction (*fig. 7*) is reproduced of a focus of caries incipiens showing its extent and the hollow in relief, reconstructed from serial sections through this focus.

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A TECHNIC FOR OBSERVING MINUTE CHANGES ON TOOTH SURFACES[1]

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Heretofore observations of minute surface changes in tooth structures have been made with the aid of several methods of varying accuracy. Visual inspection is the most primitive and inaccurate, but nevertheless most often used technic. It must be recognized that this method has many limitations and therefore is applicable, at best, only for preliminary study.

Linear, weight, or volume measurements of changes on tooth surfaces are better methods but here again many difficulties are evident. For instance, since surface changes after application of drugs or other chemicals are usually slight, measurements of dimensional changes or weight losses or gains lack accuracy. Then too, delicate allocation of changes to specific surface portions is almost impossible.

Photographic reproduction using reflected light is probably the best method thus far employed. Even with this fine technic certain difficulties present themselves. When we deal with a very smooth or a very dull surface, pictures obtained are not very instructive. It is difficult to observe small changes in the surface. In addition to this, intensity and angle of illumination are rather critical. In many instances, apparent extreme variations can be shown in the same surface merely by changing the angle of the incident light.

Another technic for observing changes in relatively thick specimens is frequently used for histologic studies. Thin ground sections are made and observed either by transillumination, or by reflected light as mentioned above. This technic is admirable when only a few sections of a given piece of material are desired. The number of serial sections that can be made from any given sample is obviously limited.

In 1940 a technic was introduced[2] which consists essentially of making a micro-impression of a surface in a transparent plastic, and viewing this impression under magnification. A similar technic, known as the "Fax-film"[3] has been suggested for the study of metallographic surfaces. The Fax-film technic is accepted by the Bureau of Standards as a method of comparing roughness of surfaces. An application of this micro-impression technic has been adapted to the study of tooth surfaces. A description of the essentials of this method follows.

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[2]Wolf, Jan, *Deut. Zahn, Mund und Kieferheilfunde*, 7: 265, 1940.

[3]Rex D. McDill, 5109 Mayfield Road, Cleveland, Ohio.

TECHNIC

A solution of a clear plastic-like celluloid in a quick-drying solvent like acetone is applied to the dry surface under study. Immediately thereafter a piece of clear plastic film is pressed onto the surface. The pressure and roll of a finger or thumb causes the plastic solution to penetrate into any irregularity on the surface. After about a minute the solvent has evaporated sufficiently to leave a tough layer of plastic firmly attached to the upper film. This film and adhering plastic are now peeled away from the sample surface. The result is a tough plastic impression which is suitable for microscopic study. This micro-impression can then be mounted on a glass slide with "Scotch tape" or else in a suitable frame as a permanent preparation.

Variations in thickness of the film allow a variable amount of light to pass through the section. This results in a pattern of ripples, waves or whatever else occurs on the surface. The darker the resulting lines, when viewed with transillumination, the deeper must have been the valleys in the surface of the specimen. In a microphotograph of such an impression, a pattern of the surface is seen with the original depressions appearing as dark areas and any projections appearing lighter than the plane of the surface proper.

It will be realized that a perfectly smooth surface will not show any discernible pattern. For this reason a smooth surface to be observed with this technic must first be caused to become irregular. The most obvious method for doing this is to etch the surface in some suitable fashion. It is advisable to use an etching fluid that will preferentially dissolve a discrete structural unit of the surface, leaving the rest of the surface unaffected, or at least relatively so.

For our study on tooth surfaces we chose to use either dilute hydrochloric acid or dilute sodium hydroxide for preferential etches. It was assumed that the hydrochloric acid would dissolve the calcified, inorganic substance, while the sodium hydroxide dissolves the non-calcified, organic portion of the tooth.

PROCEDURE AND ILLUSTRATIONS

A suitable tooth was cleansed of extraneous debris and mounted upright in an investment ring using low temperature softening impression compound. A portion of the tooth's crown was ground off, using a water-bathed emery wheel. The ensuing flat enamel surface was polished with emery paper of varying degrees of fineness until a smooth, rather highly polished surface resulted. This surface was washed with alcohol and a mark of identification made on it with a diamond point (*fig. 1*).

The specimen was then immersed in the etching solutions and the effects studied by making additional micro-impressions at suitable times. *Fig. 2* shows the same surface as in *fig. 1*, after 5 minutes in a 1.0 N NaOH solution. There seems to have been little effect. *Fig. 3* is from the same surface after 5 minutes in 0.1 N HCl solution. It is presumed here that the acid has removed a portion of the inorganic substance and the dark spots represent holes or depressions, resembling the classic structure of the enamel rods. The light areas are projections, presumably of interprismatic substance. *Fig. 4*, again, shows the same area but after treatment with 5.0 N NaOH for 15 minutes. The surface is

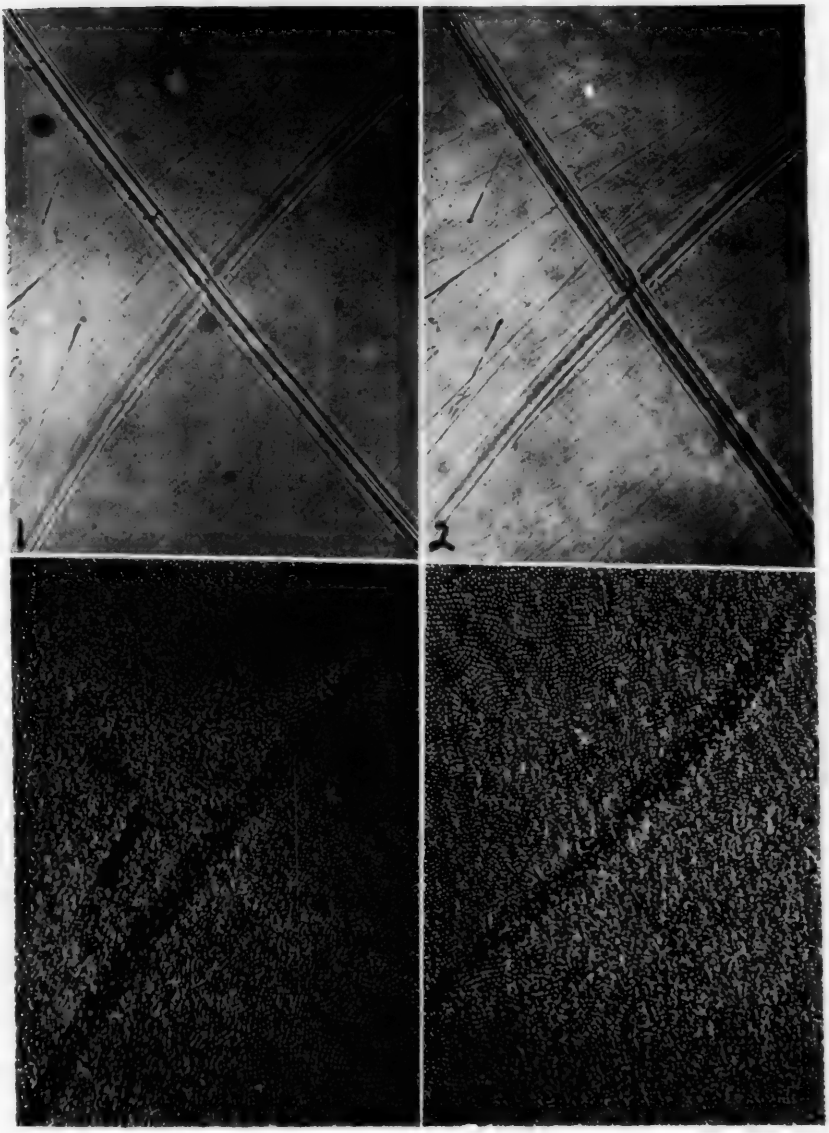

Fig. 1. Polished enamel surface marked with diamond point. Magnification 130 ×.

Fig. 2. Same surface as *fig. 1*, after immersion in 1.0 N NaOH for 5 minutes. Magnification 130 ×.

Fig. 3. Same surface as *fig. 2* after immersion in 0.1 N HCl for 5 minutes. Magnification 130 ×.

Fig. 4. Same surface as *fig. 3* after immersion in 5.0 N NaOH for 15 minutes. Magnification 130 ×.

smoother, presumably because some of the organic projections have been dissolved off.

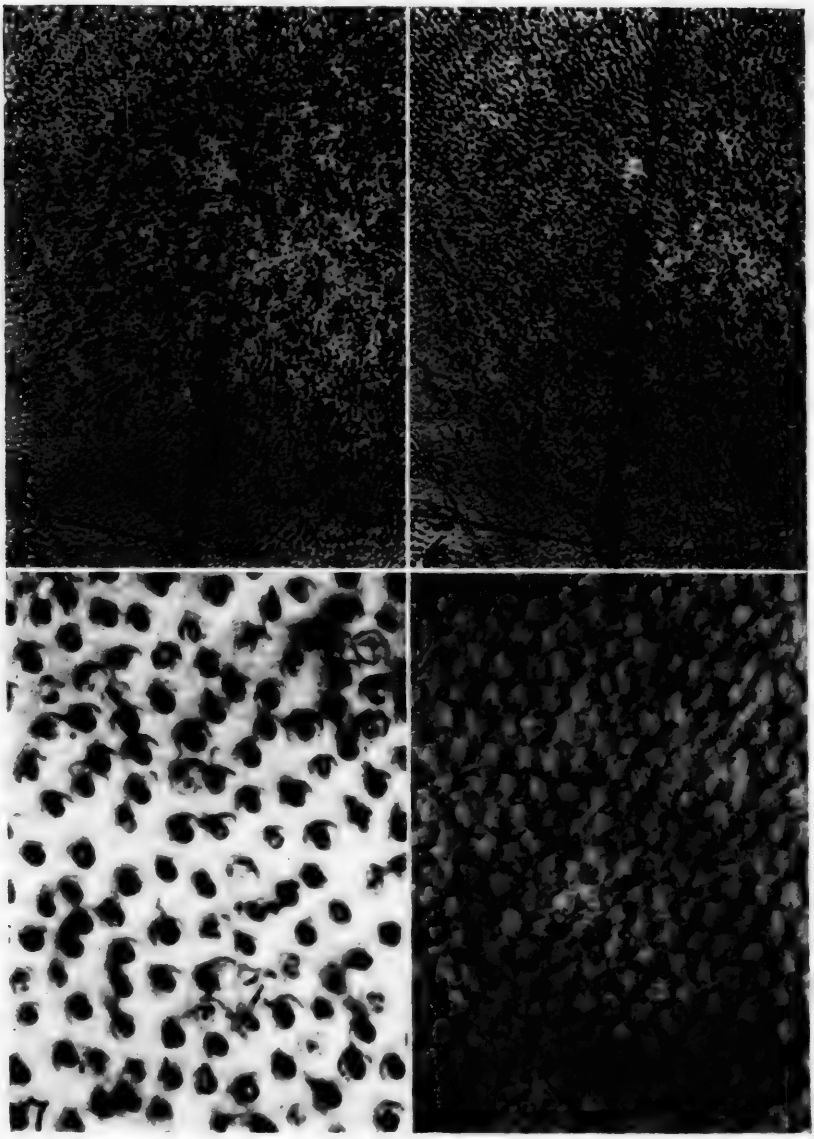

Fig. 5. Dentin surface, etched with 1.0 N HCl for 1 minute. Magnification 140 ×.
Fig. 6. Same surface as *fig. 5*, after immersion in 3 N NaOH for 15 minutes. Magnification 140 ×.
Fig. 7. Dentin surface, etched 5 minutes with 0.1 N HCl. Magnification 800 ×.
Fig. 8. Enamel surface, etched 5 minutes with 0.1 N HCl. Magnification 800 ×.

Fig. 5 shows a dentin surface after acid etching, while *fig. 6* is from the same area after sodium hydroxide. Again, a portion of the organic projections appear to be gone.

In order to indicate somewhat the order of magnification that is possible with this technic, *fig. 7* shows an acid-etched dentin area magnified 800 times. *Fig. 8* shows an etched enamel surface also magnified 800 times.

CONCLUSIONS

A technic is described which makes examination of minute surface changes on tooth surfaces a relatively simple matter. This technic can be applied in studies where drugs or other substances are allowed to come in contact with tooth surfaces. Minute changes are made very evident by taking micro-impressions at suitable time intervals. Normal surfaces can also be reproduced to give very interesting pictures, providing they are not entirely smooth.

The technic lends itself to histologic studies of hard structures, since several very thin surface films can be removed consecutively from a section, so that impressions of very thin serial sections can be simulated. A striking advantage of this technic is the exact localization of a given area of a tooth surface which can be duplicated at any desired time, since the micro-impressions are permanent and are available long after the original surface is changed.

Work is in progress in this laboratory to study the effects of drugs and other oral preparations on the human teeth, in vitro and in situ.

ADDENDUM

Since this paper was submitted for publication, our attention was called to a paper by D. B. Scott and R. W. G. Wyckoff (Public Health Reports, **61**:697, May 17, 1946), in which they describe a similar technic. Their method differs from ours in that they "shadow" collodion replicas with vaporized metal in a high vacuum. Theirs is a longer and highly complicated procedure, but the final products appear to be very similar to ours.

BANDS OF SCHREGER[1]

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The structure of enamel and the question of its development and calcification have long occupied the attention of research workers. The investigations, in recent years, on amelogenesis by Schour (1936), Schour and Kronfeld (1938), Kronfeld and Schour (1939), Schour and Massler (1940), Becks and Furuta (1939), Diamond and Weinmann (1940), Weinmann (1943) and Weinmann, Svoboda and Woods (1945), amongst several others, have directed further attention to the final product of enamel formation. In addition the nature and importance of the various markings seen in this structure are the subject of renewed inquiry. Of these, the characteristic zones known as Schreger's bands still remain unexplained and, although they have been for many years the subject of detailed study, their origin and significance still remain obscure.

In this communication the different theories concerning the bands of Schreger are briefly reviewed and certain observations on stained sections of enamel are described.

REVIEW OF LITERATURE

(a) *Nature and Origin of the Bands*

It is considered by several observers that the appearance of the bands is due to variations in direction of adjacent groups of enamel rods, some of which are viewed transversely while others run longitudinally or obliquely (the diazones and parazones of Preiswerk). Amongst those supporting this view are von Ebner (1890), Kronfeld (1937), Churchill (1932, 1935), Noyes and Schour (1938), Tims and Henry (1923), Hopewell-Smith (1913, 1918, 1926, 1927) and Beust (1934).

In a previous communication (Staz 1938) it is shown that the markings which coincide with changes in direction of contiguous groups of rods are especially prominent in stained preparations. The variation in depth and penetration of the stain suggests some difference in the degree of calcification of the enamel as postulated by Applebaum, Hollander and Bodecker (vide infra).

In an extensive investigation on the structure of enamel Pickerill (1913) makes several observations on the nature of Schreger's bands, discussing in detail the more generally accepted views on their origin. Speculating on the possibility of the bands being a simple optical effect or else an effect produced by

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the polarization of light by the enamel rods, he concluded that the most likely explanation is that the bands of Schreger "are due to effects produced by the different optical densities of contiguous groups of prisms". Pickerill found that "the prisms cut longitudinally are denser than those cut transversely, hence by reflected light the former appear white and the latter dark owing to their allowing the light to pass through them; by transmitted light. . .the exact opposite obtains. . .".

In the opinion of Mummery (1924) the bands are an optical phenomenon with but little histological significance, whereas Beust (1934) considered that their regular occurrence indicates a definite anatomic arrangement of the structural units of the enamel.

Applebaum (1933) and Hollander (1934, 1935) with Bodecker and co-workers made an exhaustive study of Schreger's bands with direct and reflected light and with soft x-rays. They concluded that the bands which had long been described as being merely an optical phenomenon are due to localised variations, of relatively small magnitude, in the degree of calcification of the enamel. This in turn may be the result of structural differences in the matrix itself but it is probable that the main factor in the appearance of the bands is the variation in optical density brought about by variations in calcification.

In connection with the calcification of enamel it may be noted that according to the more earlier accepted views, this process occurs in rhythmic cycles, the incremental lines of calcification running parallel to the striae of Retzius and transversely to the bands of Schreger. These views are strengthened by the recent findings of Schour (1936) and Schour and his associates (1938, 1939, 1940).

In modifying the usual conception of enamel formation the observations of Diamond and Weinmann (1940), Weinmann (1943) and Weinmann, Svoboda and Woods (1945) indicated that development and calcification occur in 2 phases. It is found that the first stage or formation of the matrix follows the incremental pattern. The second stage of maturation (final calcification) occurs in planes at right angles to the tooth and thus proceeds along lines running transversely to the striae of Retzius. Diamond and Weinmann (1940) found that "The calcification process begins after the entire adult thickness of the organic enamel matrix is complete, at the highest point of the morphological contour of the tooth. It then extends from the peripheral regions in the direction of the dentino-enamel junction. The incisal regions of the anterior teeth become so calcified first. Subsequently the calcification continues along the lateral surfaces in the direction of the cervical region, transversely to the incremental lines or approximately parallel to the bands of Schreger. With the posterior teeth the summit of the highest cusp elevation is similarly first calcified".

(b) *Arrangement of the Bands*

The bands are usually described as running from the amelodentinal junction towards the surface of the enamel. As shown by Hollander (1934, 1935) they lie in a direction more or less perpendicular to the enamel surface. Tims and Henry (1923) observed that they pass outwards towards the enamel surface and then

curve in their upward course slightly to the incisal edge. Churchill (1935) stated that it is possible to follow the bands from the periphery of the dentin up to the middle of the enamel section where they are lost and merge into the grey hue of the outer portion of the enamel.

Although a concentric arrangement of the bands is illustrated in the work of Hopewell-Smith (1903), the author himself made no reference in the text to this appearance. He noted, however, that the markings may be distributed anywhere throughout the thickness of the enamel but they are frequently confined to its inner aspect, particularly at the cusps of the premolars and the molars.

Fig. 1

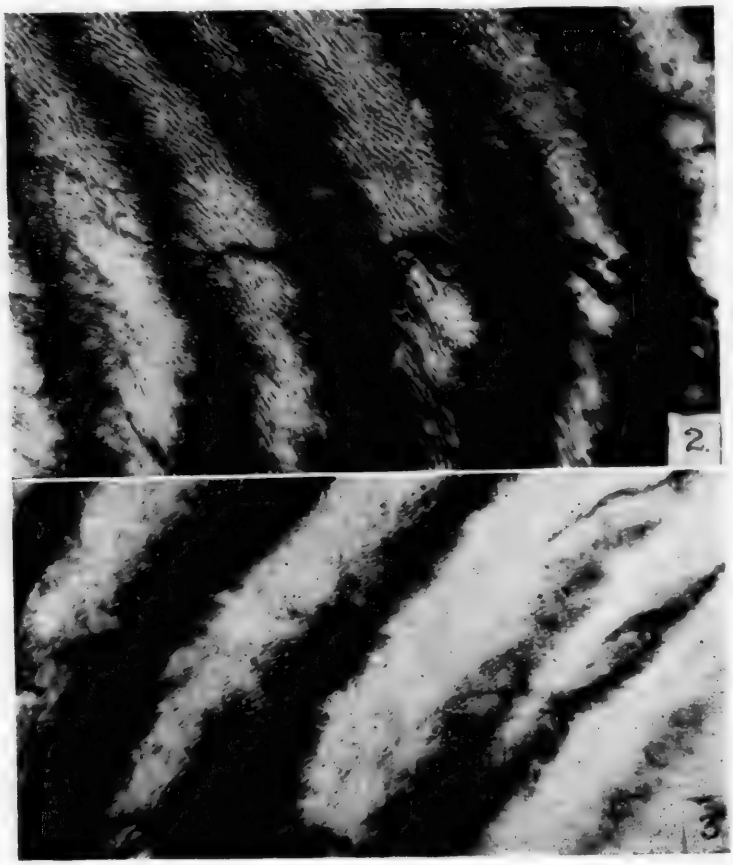
OBSERVATIONS

(a) *Nature of the Bands*

Although the staining of enamel for the detailed examination of its structure has been employed by investigators as Pickerill (1913), Hopewell-Smith (1903, 1914) Malleson (1924) and several others, a tudy of the literature suggests that most of the observations on the bands of Schreger have been made on unstained sections of enamel. Apart from the work of Hopewell-Smith (1914), Beust (1934) and Staz (1938) there is but little reference to the appearance of these markings in stained preparations.

It has been found that the bands can be clearly demonstrated in stained semi-

decalcified ground sections and if the method described some years previously is followed, (Staz, 1938) the enamel is stained in its deeper portion while the more superficial areas remain colourless. In the stained enamel the diazones stand out as distinct finger-like processes curving outwards from the amelo-dentinal junction throughout the substance of the tissue (*fig. 1*). The contrast between adjacent groups of enamel rods is illustrated in *figs. 2 and 3* where it is seen that the



Figs. 2 and 3

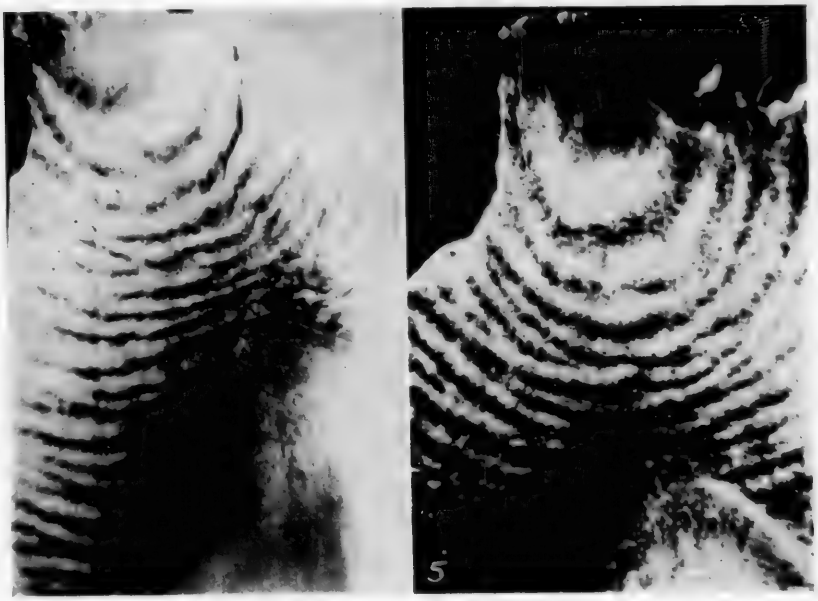
diazones are more heavily stained than the parazones. Under high magnification it is seen that the rods are less heavily stained than the interprismatic substance, while the surrounding organic sheaths are the most heavily stained of all.

If the rods are viewed transversely the deeply stained organic sheaths stand out conspicuously as dark ovals or circles. This is in contrast to their appearance in the parazones, where they appear as thin lines running parallel to the long axis of the enamel rods. When viewed collectively the prominent rings in the

diazones give rise to the dark zones which are characteristic of the bands of Schreger.

(b) *Arrangement of the Bands*

In the majority of the sections examined, the bands pass from the dentin surface of the enamel as parallel finger-like processes running approximately at right angles to the amelo-dentinal junction. This is the arrangement usually found and usually described. In one particular slide, however, a variation has been noted which merits detailed description.



Figs. 4 and 5

The specimen is a longitudinal bucco-lingual section through a lower premolar tooth. The plane of the section runs slightly anteriorly to the center of the tooth and includes a large portion of the enamel of the buccal cusp.

At the neck of the tooth the lines run out approximately at a right angle to the amelo-dentinal junction. Towards the cusp they leave at a more obtuse angle and then curve upwards and outwards as they approach the outer surface of the enamel (*fig. 1*). In their general arrangement they appear to follow a concentric pattern. This is seen very clearly in the enamel of the buccal cusp (*fig. 4*). In the unstained portion of the enamel the markings can be followed by reducing the amount of transmitted light (*fig. 5*). It is seen that the bands follow a definite spherical pattern, the centre of the sphere being situated at the highest point of the contour of the tooth. This pattern is seen in *fig. 5* and is illustrated diagramatically in *fig. 6*.

In certain areas, the bands appear to divide as they pass outwards or if they are studied in the reverse direction, adjacent bands merge as they approach the inner aspect of the enamel. This is illustrated particularly in *figs. 1, 2 and 3.*

Fig. 6

DISCUSSION

(a) *Nature and Origin of the Bands*

In stained enamel the bands are found to coincide with changes in direction of adjacent groups of rods, the diazones being more prominent and more heavily stained than the parazones. Although the deeper staining of the diazones suggests variations in the degree of calcification of the enamel as indicated by the findings of Applebaum (1933), and Hollander (1934, 1935), it is found that the apparent difference in intensity of staining depends on whether the rods are observed in cross section or longitudinally. In the diazones they are viewed transversely and here the stained organic sheaths surrounding the enamel rods stand out prominently as darkly stained circles or ovals, which when seen collectively, show up in strong contrast to the relatively thin parallel lines seen in the parazones.

Similarly in unstained enamel, it is seen that the parazones and diazones appear as bands of different degrees of light intensity, as pointed out by several

investigators. It is evident that this variation in intensity is associated with the effect of the organic sheaths on light transmitted through or reflected from the enamel rods, and it is postulated that these organic sheaths are associated with the different optical densities of contiguous groups of rods described by Pickerill (1913).

(b) *Arrangement of the Bands*

While leaving the inner surface of the enamel at approximately a right angle to the amelo-dentinal junction, the bands tend to follow a concentric arrangement. This spherical pattern is not always present, depending on the manner in which the enamel is prepared. If the plane of the section does not run exactly through the center of the sphere the concentric appearance will not necessarily be seen and the more usual pattern appears.

It is apparent, therefore, that the parazones and diazones are laid down in a concentric spherical pattern, the centre of the sphere being situated at the highest point of the contour of the tooth and although it would seem that this is the normal arrangement it has failed to excite comment by previous investigators. Apart from the illustration of Hopewell-Smith (1903) there is no apparent reference elsewhere in the literature to a concentric arrangement of the bands. It is possible that this has always been accepted as being self evident, for as Robinson (1945) observes, "It seems quite obvious that structures such as Schreger's lines would look like the specimen illustrated if the material was not cut directly through the long axis of the tooth". Nevertheless, it is considered that the occurrence of the bands in a spherical pattern warrants mention. Attention is therefore directed to this feature in the structure of enamel.

(c) *Significance of the Bands*

While several authorities consider the bands to be an optical phenomenon, without histological significance, others ascribe a more important role to these structures.

It has been shown that they coincide with the tortuous course followed by groups of enamel rods. This is a guide to the strength of the enamel as indicated by Hopewell-Smith (1913, 1918, 1926, 1927) where the greater the number of Schreger's bands the more complex is the arrangement of the rods and the stronger is the structure of the enamel.

In addition, it has been shown that the center of the spherical pattern in which the bands are laid down is situated at the highest point of the contour of the tooth. This is the same region where, according to Diamond and Weinmann (1940) maturation of the enamel matrix commences.

SUMMARY AND CONCLUSIONS

The bands of Schreger have been described, especially as seen in stained sections of enamel.

The view that the appearance of the bands is due to alternation in position of adjacent groups of enamel rods (diazones and parazones of Preiswerk) is confirmed.

The presence of the organic sheath enclosing the enamel rods has been shown to be associated with the appearance of the bands.

Attention is directed to the concentric or spherical pattern in which the bands occur, the center of the sphere being at the highest point of the contour of the tooth. Until their origin and development are fully understood, no definite interpretation can be placed on the presence of the bands or their significance in fully formed mature enamel. It is suggested that they may indicate stages in the calcification of enamel if the more recent views on amelogenesis are accepted.

The author wishes to express his thanks to Miss J. P. Grant, B. Sc. (Hons.) and to his wife for valuable assistance in connection with the preparation of this communication.

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ODONTOBLASTS AND DENTINOGENESIS[1]

(A Histochemical Study)

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INTRODUCTION

The early studies of Robison and his co-workers in which alkaline phosphatase was shown to be present and causally related to calcifying tissues has been fairly well established (1). Within the past decade, methods have been developed which now permit the histochemical localization of the phosphatases (2). Since these methods have developed, considerable literature has appeared which deals with the histochemical localization of these enzymes in various tissues and organs.

In regard to the localization of phosphatase in teeth, Robison and Somes (3) reported considerable amounts of this enzyme in the rapidly growing teeth of young rodents. Kabat and Furth (4) illustrated sections of the enamel organ of the rat in which phosphatase also appears to be present in several of the components. Horowitz (5) contended that alkaline phosphatase is present in both ameloblasts and odontoblasts of young teeth. Gomori (6) likewise remarked on the presence of this enzyme in the various components of the developing tooth. Finally, Bevelander and Johnson (7) described the histochemical localization of alkaline phosphatase in the developing tooth of the pig, and showed among other things that this enzyme is present in odontoblasts before and during early calcification of dentin. Recently, Gottlieb (8), stated that the function of odontoblasts appears to be 'superfluous', but in view of the fact that the odontoblast complex: cell, process and tubule, have persisted throughout phylogenetic development, he has introduced a speculation in which a correlation is proposed to show that the function of the dentinal tubules is related to the mechanics of enamel attachment.

In this paper we wish to present the results of our studies on the development, and to some extent the function of the odontoblast and the dentinal fibril as revealed by histochemical methods.

MATERIAL AND METHODS

The material used in this investigation consisted of embryos of freshly slaughtered pigs. In those stages which permitted sectioning without decalcification, sections were made in the usual manner. The teeth of older embryos, and of mature rats and guinea pigs were sectioned by grinding.

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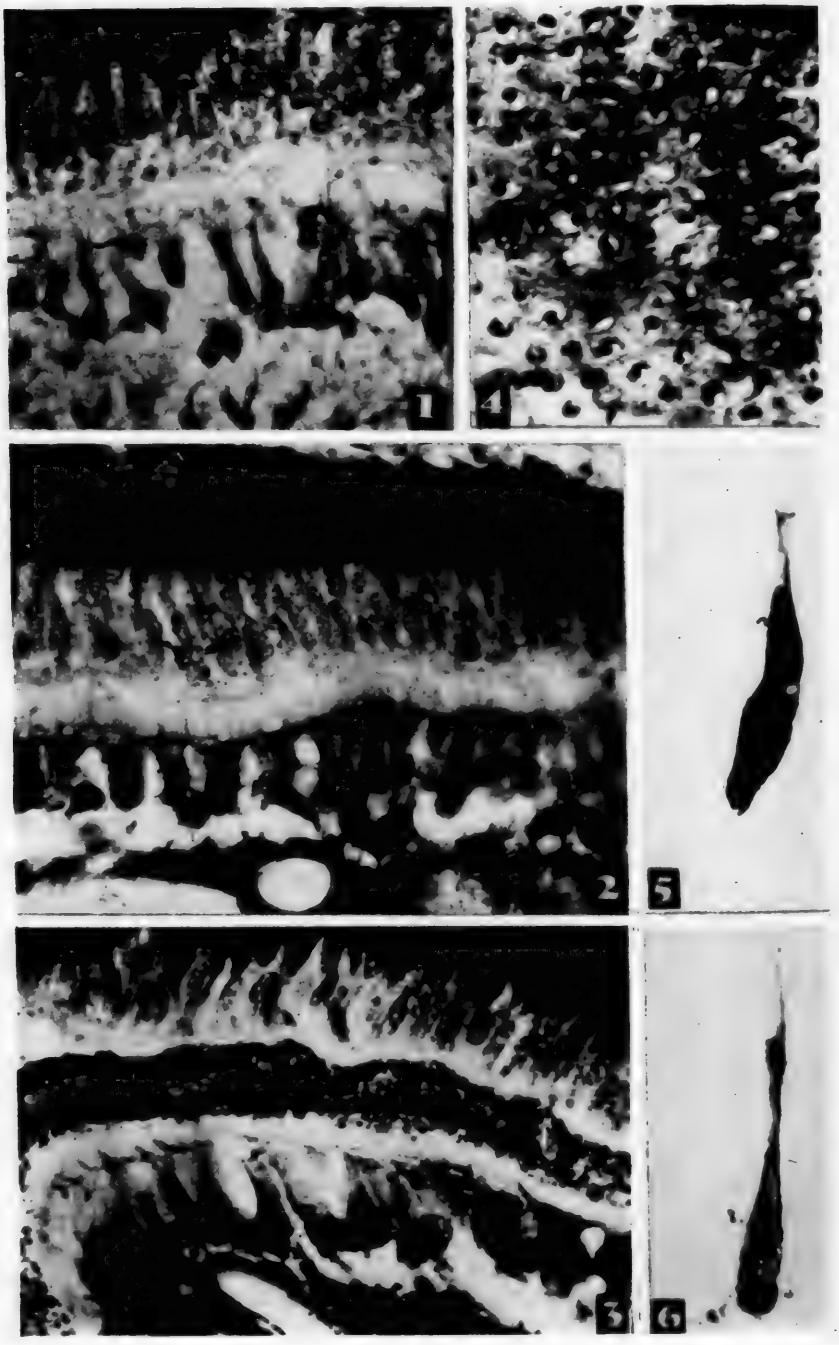

Figs. 1–6

Localization of alkaline phosphatase was determined by the method described by Gomori (2). When ground sections of dentin were to be studied, 2 control tests were run simultaneously. The first consisted of no treatment at all, the second was treated in the manner in which the usual control sections are prepared. These specimens were mounted in very thin clarite to ensure penetration of all spaces in the dentin.

OBSERVATIONS

We have divided the development of the odontoblasts into 3 arbitrary stages as a matter of convenience for purposes of description.

First stage. The first stage of development as shown in *fig. 1*, consists of typical stellate cells lying below the odontoblastic zone. In the apical part of the section shown in this figure, a number of loosely arranged cells which have just begun to assume a modified columnar form are present. These cells now show for the first time, a definite nuclear orientation. At the free surface of the layer of cells which are to be the future odontoblasts, we also observe the delicate irregularly arranged dentinal fibrils lying in the space which soon comes to be occupied by predentin. At this stage of development the phosphatase localization in these incompletely differentiated cells is confined for the most part to the nucleus.

Second stage. The intermediate state of differentiation of the odontoblast is represented in *fig. 2*. In this stage the odontoblasts have assumed a definite orientation. The cells are columnar in shape, the nucleus is definitely oriented and Tomes' dentinal fibrils lie within a zone of predentin. Reference to this illustration shows that during this phase of development, the odontoblasts and the fibrils are strongly positive (darkly stained) in regard to phosphatase localization.

Third stage. The third stage we have chosen to illustrate consists of the cellular complex which appears when active calcification of dentin is taking place. *Fig. 3* shows again that during this stage of development, the odontoblasts and the dentinal fibrils contain considerable amounts of phosphatase.

Older dentin. In order to ascertain the histochemical reaction of the dentinal fibrils in older dentin, methods of preparation other than the usual sectioning procedures were used. In these specimens we prepared ground sections and also teased the odontoblasts and their processes away from the dentin.

The reproduced photographs represent sections of selected regions of teeth in which the darkened areas represent the histo-chemical localization of alkaline phosphatase.

Fig. 1. Strip of developing tooth showing at the top, ameloblasts, a relatively clear space in which Tomes' fibrils appear and below this, differentiating odontoblasts.

Fig. 2. Comparable area of developing tooth as shown in *fig. 1*. Odontoblasts now columnar, nucleus, cytoplasm and fibril are phosphatase positive.

Fig. 3. Region of developing tooth showing strip of calcified dentin. Odontoblasts and subjacent tissue contain considerable amounts of the enzyme.

Fig. 4. Transverse section (ground) of well calcified dentin. Note intense blackening of dentinal fibrils.

Figs. 5 and 6. Two odontoblasts with part of fibril. These cells were teased away from dentin. Entire complex phosphatase positive. Note also darkened supra-nuclear area in odontoblast shown in *fig. 6*. This is probably the Golgi apparatus.

a. Ground sections: The reactions observed in the control ground sections show an intense calcium deposit on the wall of the tubules, but not of the dentinal fibril. Longitudinal sections of ground specimens are not as suitable for observation as are transverse sections due to the difficulty of maintaining the flat field necessary for observation under high magnification. In transverse test sections of older dentin we repeatedly observe a very intense blackening of the fibril which we believe to be due to phosphatase activity (*fig. 4*).

b. In a series of preparations in which we teased the odontoblasts and their processes away from the dentin, we were able to ascertain by the histochemical method that the entire odontoblast complex is phosphatase positive (*figs. 5 and 6*).

DISCUSSION

In a previous report (7), we described the localization of alkaline phosphatase in odontoblasts just prior to and during the early calcification of dentin. In this respect our work confirmed the observations of previous investigators in a number of details (2–6). We (7) made the tentative suggestion that in view of the established correlation between the presence of this enzyme and the well known role it plays during calcification, the probable function of the odontoblast was to mediate or transfer phosphatase which in turn results in the phosphate metabolism upon which calcification is dependent.

The conclusions reached by V. Korff (9) and Orban (10) in which these investigators maintain that the odontoblasts are not concerned with the calcification of dentin, were based upon routine histological preparations, before the present histochemical methods were available. In the light of these facts, we may call these interpretations of the function of odontoblasts incomplete rather than completely erroneous.

Gottlieb (8) has proposed that the function of the odontoblasts is to produce a 'showering effect' upon the enamel, which in turn, in some manner not understood by the present writers, helps to maintain contact between the enamel and the dentin. It appears that in view of the available evidence (2–7) pertaining to studies bearing on the topic of dentinogenesis, any statement regarding the function of odontoblasts must of necessity take cognizance of histochemical studies. The disregard which Gottlieb (8) has shown for the vast armamentorium of general and specific biochemical information which the histologist now has available, tends to reduce our subject matter to a very tenuous rather than a sound, though necessarily changing scientific basis.

The results of this present investigation has been to confirm and extend our previous histochemical studies relating to dentinogenesis. In addition to a detailed study concerning the differentiation of the odontoblast and the localization of phosphatase in these cells, we have been able to ascertain that the dentinal fibril is also strongly phosphatase positive even when it lies deeply embedded within well calcified dentin.

In view of these findings we can now be reasonably certain that the odontoblast and its process have a definite metabolic function and that this function is specifically correlated with either the production or transfer of alkaline phos-

phatase to the area within the dentin where phosphate is split and eventually precipitated to form the calcified matrix of this tissue.

SUMMARY

1. During the differentiation of the odontoblast, alkaline phosphatase comes to be localized within the nucleus, cytoplasm and the dentinal fibril just before and during the early calcification of dentin.

2. In older dentin, this same situation holds true and in addition, Tomes' dentinal fibril is also strongly phosphatase positive.

3. On the basis of the well known function of phosphatase in connection with normal calcification, we can now state with a fair degree of certainty that the function of the odontoblast and Tomes' fibril is to a great extent biochemical—to produce and mediate phosphatase transfer which in turn leads to the splitting of phosphate in the very region in which we know it is deposited.

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FURTHER OBSERVATIONS ON VITAL STAINING OF DENTIN AND ENAMEL[1]

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Investigators (1) reporting their recent work on the vital staining of dentin and enamel, deny the existence of currents of dental lymph. Their denial may serve to further the acceptance of physiological changes in the dentin, because a renewed discussion may well clear up some obscure points and further demonstrate the presence of a two directional flow of dental lymph[2] in both dentin and enamel. Our observations to date are based on the examination of 83 stained teeth of man, 104 of dog, 14 of monkey, 16 of sheep, 6 of calf, and 1 of cat.

The average reader, not particularly interested in the specialized field of histology, may think that the subject under discussion is of only theoretical value. This is not true; it has a far greater significance. The determination of whether or not metabolism occurs in the dentin has a bearing on some phases of operative dentistry and also may be of prime importance in the problem of dental caries immunity.

One phase of caries susceptibility or immunity may be associated closely with as yet unidentified products in the dental lymph. The concept of a fluid in the dentin is by no means new; it dates back to 1854 when Koelliker (2) stated "During life the canals" (dentinal tubules) "contain a clear fluid and they cannot therefore readily be detected in recent preparations, it is less difficult in dry sections, when they become filled with air, and appear separately as black lines by transmitted light, and by reflected, as silvery threads." Since that time similar statements have been repeated without the presentation of proof.

The first evidence (3) of the truth of Koelliker's contention came in 1893 when a ground section of a tooth with an amalgam filling was examined (*fig. 1*). It was shown that the oxides of the filling had been distributed along the adjacent dentinal tubules, thus demonstrating what was probably the first vitally stained tooth. Although this is from an old wood cut, comparision with *fig. 5* shows the great fidelity and detail in reproducing the specimen seen under the microscope. Beust (4) injected dyes by means of a hypodermic syringe through the apical ends of extracted teeth, thus demonstrating a variable permeability of the dentin. He mentioned the possibility of a lymph supply to the enamel. Later, (5) histological evidence was presented, showing the presence of fluid in dentin by

[1] Investigation partly financed by the Hans Eliot Fund. Received for publication April 8, 1946. Revised by authors, August 20, 1946.

[2] Some histologists prefer the term "tissue fluid".

observing gas bubbles within the dentinal tubules. The fluid had been previously named "dental lymph" (6).

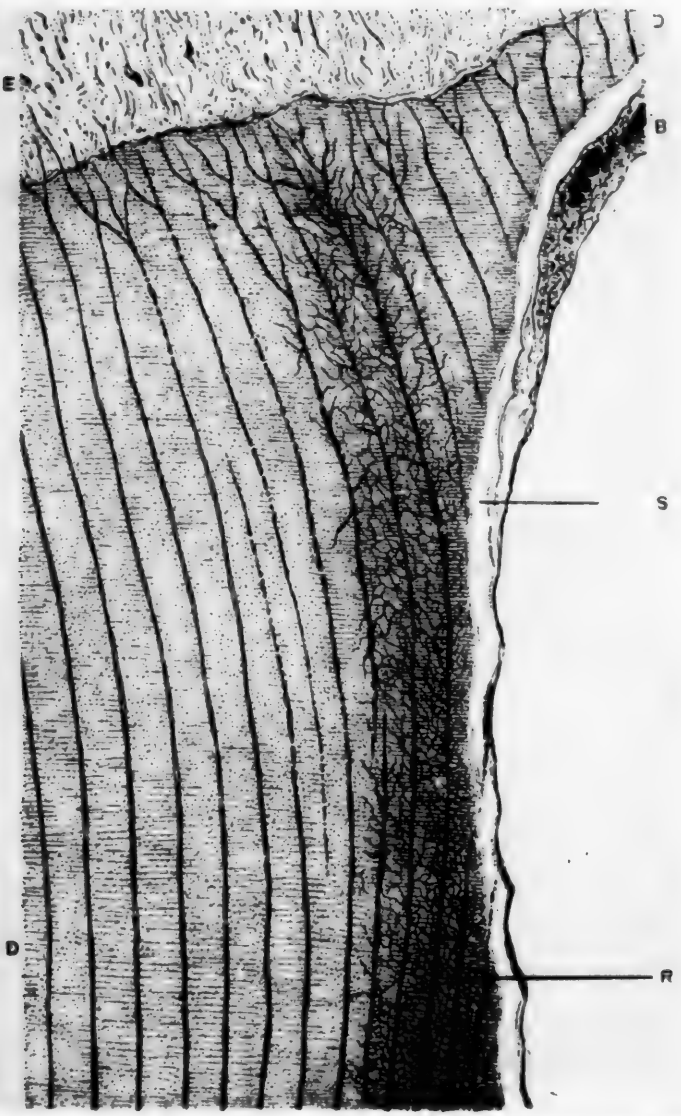

Fig. 1. Vital staining of dentin (R) by amalgam filling (C. F. W. Bodecker, 1893). Correctness of detail of lateral branches is established by comparison with *fig. 5*.

Zander and Smith (1), as a result of experiments performed on the teeth of dogs and human beings, reach the following conclusion: "1. The so-called "dead tracts", "metamorphosed dentin", "calcific barrier", and irregular dentin are

penetrable by silver nitrate solutions. 2. There is no appreciable difference in the penetration of silver nitrate solution into dentin which has undergone degenerative changes as compared to the penetration into sound dentin of pulpless teeth. The penetration of silver nitrate solution into dentin is a phenomenon not necessarily associated with the flow of a tissue fluid. 3. The penetration of silver nitrate solution cannot be used as an indicator of dentin metabolism".

The experimental methods (1) employed are well planned and the number of teeth used are sufficient with exception of those in the pulpless teeth group. We agree, to a certain extent, with their statement in the first paragraph of their conclusions. However, we disagree completely on the 2 points stated in the second and third paragraphs of their conclusions. Briefly detailed experimental evidence follows in support of our conclusions.

The concept of diffusion of dental lymph (tissue fluid) was not based upon the phenomena of vital staining by silver nitrate alone. Our conclusions (7) were drawn from observations made on the diffusion not only of silver nitrate, but also of water soluble eosin, congo red, borax carmine, methylene blue, argyrol and potassium permanganate. Our work also included the use of India ink previously recommended by Fish (8).

The above stains, all water soluble except India ink, were inserted in a dry state and their diffusion proved the presence of a fluid (dental lymph) in the dentin. The first observations of vital staining were made on ground sections; later studies were made of decalcified sections of those teeth in which argyrol and silver nitrate were used. The fluid which dissolved the water soluble dyes, normally present in young dentin, occurs not only in the tubules but also in the matrix. When water soluble dyes dissolve in the dental lymph, the dentin becomes stained fairly homogeneously without leaving any dye particles. The dye particles in the dentinal tubules have the appearance of a suspension in a supersaturated solution. This observation applies also to India ink. In the absence of any movement or current of the fluid, the undissolved stain particles could not be distributed throughout the dentin but would remain concentrated close to the site of their deposition in the stain pocket or cavity.

Further reference must be made to the use of argyrol, a silver proteinate. It was selected at the advice of Dr. Charles C. Lieb, recent Head of the Department of Pharmacology, as being particularly adapted for this study. It is a non-ionizable substance, and as such, is unlikely to enter into combination with other substances found in the dental tissues. The argyrol stain particles appeared in suspension in the dental lymph and their subsequent distribution throughout the dentin demonstrated movement of this fluid.

The stain particles found in specimens treated with silver nitrate probably are also a silver proteinate; its use produced similar phenomena. It was therefore stated that "silver stains such as argyrol and silver nitrate were found to be equally illuminating in both ground and decalcified sections". (10). Ground section studies were the basis for the original report (7). Further observations demonstrating movement (current) of the fluid (dental lymph) are discussed in greater detail below.

The first misconception (1) concerns the permeability of metamorphosed

dentin ("chromophobic" dentin; (11) "dead tracts" (8)). Permeability is a matter of degree; our observations, frequently reiterated, have shown that permeability is reduced in metamorphosed dentin. The following quotations will bear out this statement: "Maturity, abrasion or caries greatly reduces the permeability of the dental tissues" (7). "That secondary dentin not only protects the pulp against exposure by abrasion but that it also retards the progress of caries by making the dentin less permeable" (5).

Beust (11) in speaking of chromophobic dentin areas which do not take stain: "In this obliteration process, the permeable, sieve-like structure of the dentin is converted into a tissue, which according to Miller, exhibits resistance to acids and, according to my staining experiments, refuses the ingress of ordinary staining reagents".

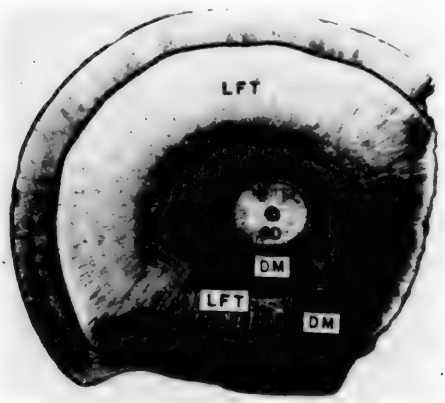

Fig. 2. Ground, transverse section of tooth of a dog though original pulp chamber. Tooth showed marked attrition causing extensive but incomplete dentin metamorphosis (DM). The light, translucent tubules are lymph filled (LFT), hence permeable. SD= Secondary dentin. (215×)

Fish (8) made the following observations: "The odontoblasts associated with injured tubules lose their fibrils and degenerate. Many of them die and disappear. The remainder show degenerative changes and return to normal, laying down the tubules in the secondary dentine".

It is clear that Beust and Fish appeared to hold opposite views. The latter regarded dentin subjected to the external irritation of attrition or caries as possessing a variable permeability, while Beust believed it impermeable.

Transverse sections of metamorphosed areas are more instructive than longitudinal sections in demonstrating their permeability. In longitudinal section, the area between pulp and incisal tip appears densely metamorphosed, whereas in transverse section the dentin is not uniformly opaque; some translucent zones may be seen. *Fig. 2* shows a transverse section of a canine tooth of a dog in which the dentin had been exposed by attrition. These translucent areas, composed of fluid (dental lymph) filled tubules which, being permeable would

allow stains to penetrate to the pulp. The metamorphosed areas (DM) show many translucent tubules (LFT) which indicate that a certain degree of permeability still exists. Secondary dentin (SD) has been formed in which some tubules are metamorphosed, others remain filled with lymph, hence it has a greatly reduced permeability. All tubules in longitudinal sections of metamorphosed dentin appear opaque, because in a section of 200 microns, the observer is attempting to look through layers of approximately 30–50 tubules making some translucent fluid (dental lymph)-filled tubules invisible. This is particularly true when a fairly low magnification is used with a focus which encompasses the entire thickness of the section.

To sum up the first phase of this discussion: Fish (8) and the authors agree that "dead tracts", "metamorphosed dentin", "sclerotic barriers" and irregular dentin are permeable, but that permeability is greatly reduced over that observed in dentin of young teeth.

An observation (1) which leads to completely erroneous conclusions concerns the reaction of pulpless teeth. A statement is made that: "The fact that the silver nitrate penetrated through the dentin of pulpless teeth and the fact that silver nitrate penetrated into tubules which were not in communication with the pulp further indicates that living processes are not an essential factor in the penetration of silver nitrate". It is regrettable that neither the time which elapsed after pulp extirpation nor the age of the teeth was mentioned. Both these factors affect greatly the permeability of the dentin (12, 7). Also, their conclusions might have been different if they had taken into account our observations that: "The removal of the dental pulp temporarily increases the permeability of the dental tissues, indicating that this organ is active in regulating the diffusion or the tissue fluid" (7). This observation also usually applies to dentin zones cut off from the pulp by cavity preparation, particularly if the tooth is that of a young person.

There is a marked difference between the distribution of dye particles in vital teeth and teeth with pulps that have died recently. In vital teeth, the dye is distributed in definite channels, i.e. mainly within the tubules. On the other hand, in teeth with recently devitalized pulps the dye dissolves in the dental lymph which is no longer organized according to the tubules. In this case a simple diffusion occurs. The dye passes in all directions, not only along the dentinal tubules but also across their axes, demonstrating loss of organization. Recently extracted, unfixed teeth act in a similar manner when stained in vitro.

The work from which the above conclusions (7) are quoted was done on teeth of animals and human beings. Subsequently, 33 more human teeth were prepared by Drs. Ziskin and Zegarelli. The latter group included 1 patient who had 2 remaining mandibular canine teeth which were to be removed to prepare the mouth for a full denture.One of these teeth had a history of previous pulp devitalization (approximately 5 years). Two-thirds of the old root filling was removed, the canal reamed and Gentian violet sealed in with oxyphosphate cement. The pulp was extirpated in the other tooth and the apical third of the canal filled immediately with gutta percha. Then Gentian violet was likewise

sealed in with cement; both teeth were removed after 36 days. Ground sections of these teeth showed (1) deep dye diffusion with evidence of stain in the enamel and cementum, of the tooth with the recently devitalized pulp and (2) stained only in the neighborhood of the pulp canal of the other tooth. Our conclusions (12) were that: "1. Vital teeth are less permeable than teeth immediately after pulp extirpation. 2. The permeability status of pulpless teeth changes. Immediately after pulp extirpation, the permeability of the tooth is highest, reducing slowly thereafter".

We do not concur therefore, with our critics that the penetration of dyes through pulpless teeth or dentin zones cut off from the pulp by cavity preparation precludes the possibility of metabolism. In this respect the opinions of physiologists are pertinent. Osterhout (13) for instance stated that: "It is well known that death is accompanied by an increase in permeability. Thus a slice of red beet kept in water will live for a long time without giving off pigment: but as soon as it is killed, the color begins to escape from the cells. . . . It is a matter of common observation that cells may resist the penetration of certain dyes as long as they are alive, but absorb them readily as soon as they are killed". Thus the augmented dye diffusion through pulpless teeth does not disprove dentin vitality; on the contrary it appears to be one of the proofs of vitality of this structure.

We wish to present further evidence of a two-way current of the dental lymph (tissue fluid). Cutting into dentinal tubules and sealing a solid dye in the dentin results in its diffusion, both toward the pulp (afferent) and to the periphery of the tooth (efferent). Our present knowledge of the increased permeability of dentin when cut off from the pulp, such as occurs in teeth with devitalized pulps, might cause some doubt concerning both the efferent and afferent flow of dental lymph, were it not for supporting observations.

On the basis of past observations, a diagram (*fig. 3*) was made which indicates the afferent currents of dental lymph. The technique of vital tissue staining as used in our experiments made it necessary to adopt the term "secondary vital staining", which occurs through a redistribution of the dye by the pulp. This takes place when zones of dentin, sometimes including enamel, are stained by a dye which has passed from a dentin stain pocket (*fig. 3 SP*) to the pulp and then has been redistributed to other areas of the tooth (secondary efferent staining SES), not in tubular connection with the stain pocket.

Fig. 4 is of one of the specimens offered in support of the diagram (*fig. 3*). Many of the first series of vitally stained teeth showed no secondary vital staining. The reason for the absence of this phenomenon probably is the length of time which was allowed to elapse after placing the dye and the removal of the tooth which in some instances was 4 weeks. This time interval was reduced radically, to even as short as 6 minutes. The tooth in *fig. 4*, was stained for 6 hours. This shows a lingually placed stain pocket (SP) filled with argyrol in the dentin of a second incisor of a 17 year old youth. During a period of 6 hours the dye was dissolved and passed through the dentin into the pulp, was then dis-

tributed throughout this organ. It then passed into the dentin (SES), and penetrated the enamel as observed in the organic matrix. As the stained dentinal tubules (SES) were far removed from the stain pocket (SP(and uninjured by the operation, we believe that this observation demonstrates the efferent current of

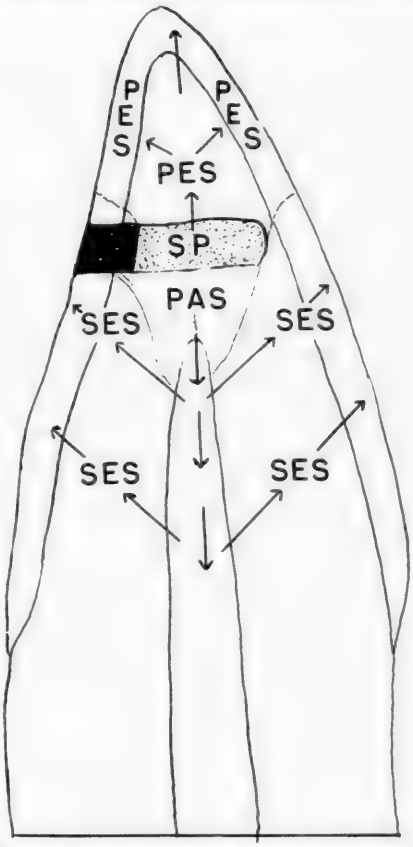

Fig. 3. Diagram of Lymph Currents shown by Vital Staining. Dye passes from stain pocket (SP) toward pulp by afferent current causing primary afferent stain (PAS). Dye is then redistributed by pulp resulting in secondary efferent staining (SES) of dentin and enamel. Also, dye is passed from stain pocket (SP) to tip of tooth through dentin and enamel causing primary efferent staining (PES).

dental lymph by means of secondary vital staining which was not observed by the other workers (1).

Fig. 5, a photomicrograph of a human tooth stained with argyrol, shows both primary afferent stained (PAS) and secondary efferent stained areas (SES). The dentin, stained by the dye from the pulp (below) has most of the argyrol collected above (SES), near the deeply stained enamel matrix (M).

A comparison of this photomicrograph with the wood cut in *fig.* 1, shows the exactness of the latter in depicting the stained lateral branches of the dentinal tubules.

Doubt also might be cast on the presence of an afferent current of dental lymph due to injury of the dentinal tubules by drilling the experimental cavity. Even if only the terminal ends of the dentinal tubules are opened in order to place a dye deposit, the criticism might again apply that these could be regarded as injured and abnormal, thus hastening dye diffusion as was shown to be the case in pulpless teeth. Evidence of an afferent flow of dental lymph was ob-

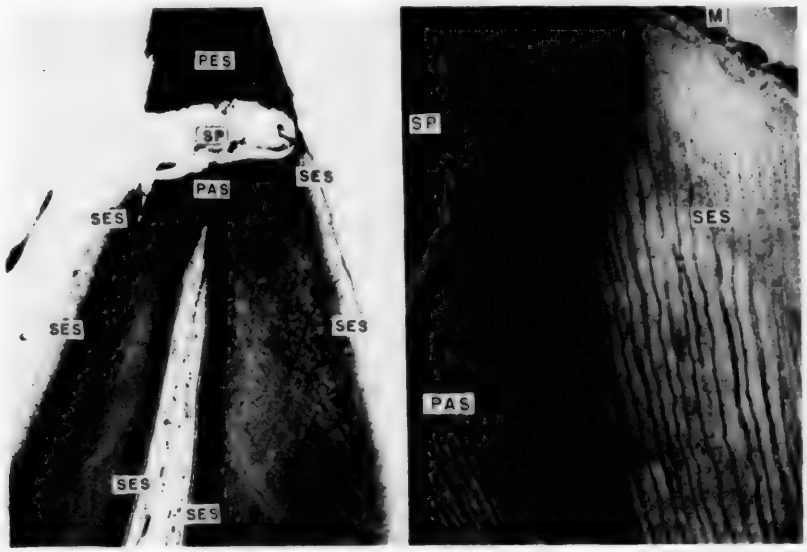

Fig. 4. Second incisor of youth, age 17, stained vitally with argyrol for 6 hours. PAS=primary afferent stain from pocket (SP) to pulp; SES=secondary efferent stain from pulp through dentin into enamel; PES=primary efferent stain passes from pocket through tip of tooth (30× mga.)

Fig. 5. Human tooth stained with argyrol. PAS=primary afferent stain passes from stain pocket (SP) toward pulp. SES=secondary efferent stain is transmitted from pulp below, through dentin to enamel matrix (M). (540×)

tained as a result of another experiment (14). It was decided to test the permeability of the surface of the enamel, employing the following technique: metal veneer crowns were prepared for the canine teeth of a dog, filled with the dye, potassium permanganate, and attached by oxyphosphate cement in the cervical region. The results indicated that the normal enamel surface is impermeable (14) but the slight decalcification caused by the phosphoric acid in the cement, allowed the potassium permanganate (*fig. 6* PP) to penetrate the enamel (E) and the dentin (D). It was impossible to determine whether or not the dye reached the pulp, because decalcified sections could not be made of these specimens, owing to the fact that potassium permanganate is destroyed by acid.

The fact that the dye penetrated from the exterior is proof of an afferent current of dental lymph, as the dye passed from the enamel into the dentin through uninjured dentinal tubules.

No mention has been made of the staining of the enamel by way of the pulp. *Fig. 7* is a section of a vitally stained human tooth showing argyrol particles in the enamel matrix (E). Most of these are large and are located in or on the

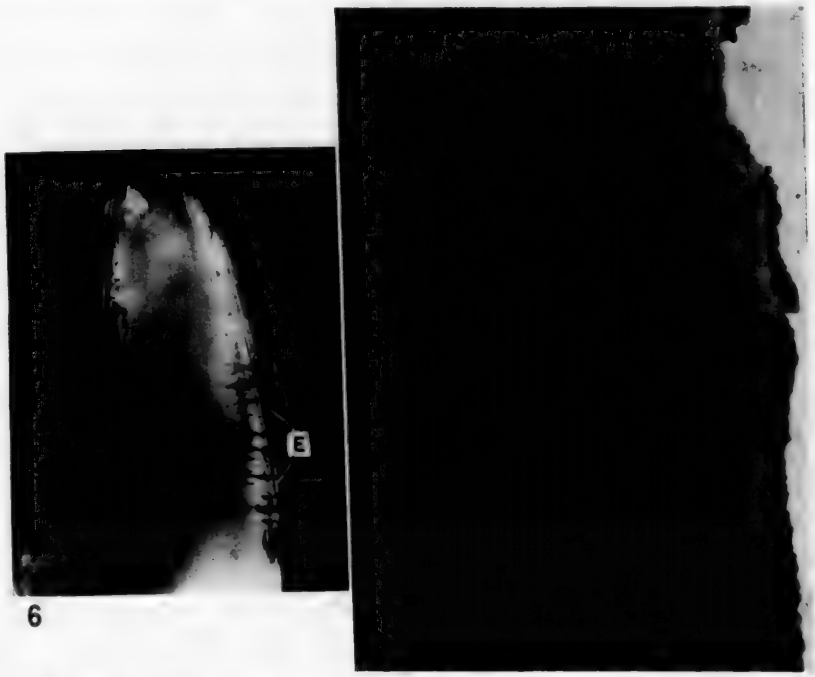

Fig. 6. Metal veneer crown with potassium permanganate was placed on tooth and attached with dental cement only at cervix. Phosphoric acid of cement caused slight surface decalcification of enamel (E) allowing dye (PP) to penetrate enamel and dentin (D). Photographed by reflected light.

Fig. 7. Zone of secondary efferent stain containing large and medium particles in dentin (D) and enamel (E). Enamel cuticle is loaded with dye.

enamel cuticle. Emphasis is placed on the fact that the stain penetrated the enamel from tubules not in connection with the stain pocket.

A final topic must be mentioned: the varying size and location of argyrol particles in vitally stained dentin and enamel (*figs. 8–11*). The particles can be classed roughly as large, medium and minute. The large particles range from 6 to 11 microns in length and 4 to 9 microns in width; the medium, 3 to 7 microns and the minute, round ones, between .3 to 1 micron in diameter. *Figs. 8–11* demonstrate the varying location and size of argyrol particles. These conditions are fairly constant in relation to lymph current direction. The fact

that formalin, used in fixing specimens, does not precipitate argyrol, suggests that varying particle size and differences in location may throw some light on the

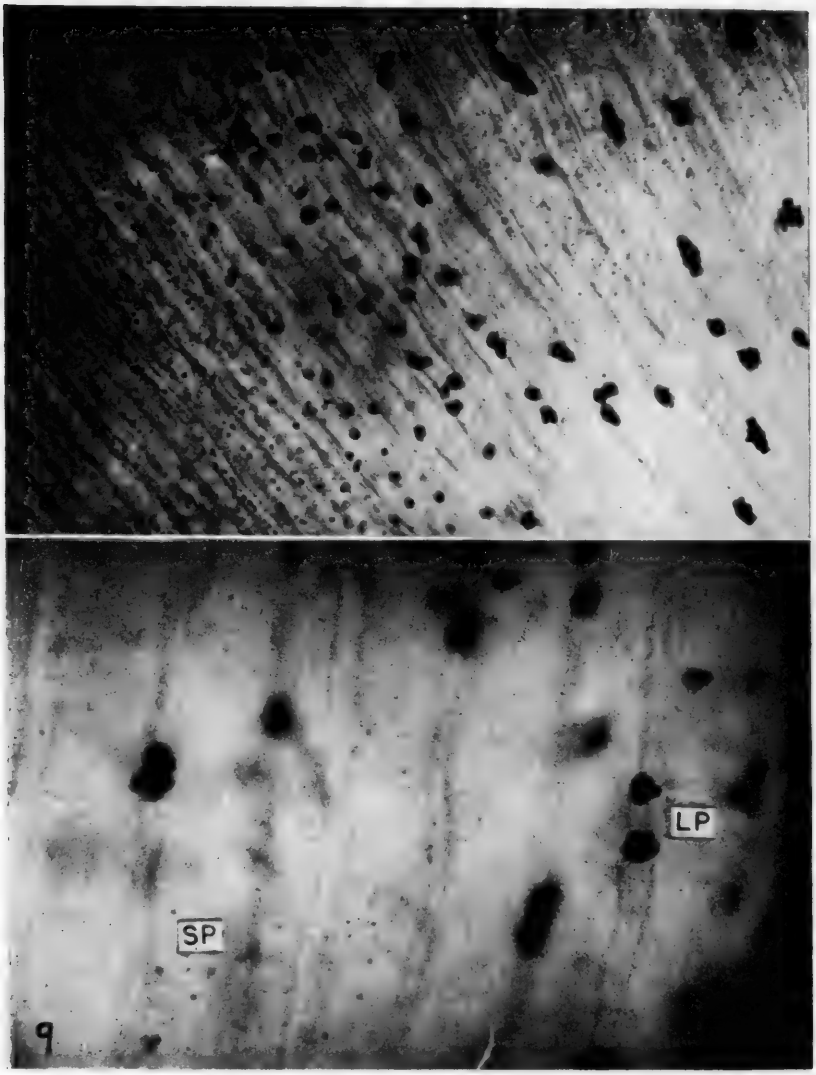

Fig. 8. Argyrol stained dentin in efferent zone showing large and medium particles in tubules and small ones in matrix. (2700×).

Fig. 9. Secondary efferent stain in dentin filled with large particles of argyrol (LP) and small ones (SP) in matrix (4500×).

character and concentration of the dental lymph. Work is being continued in this field.

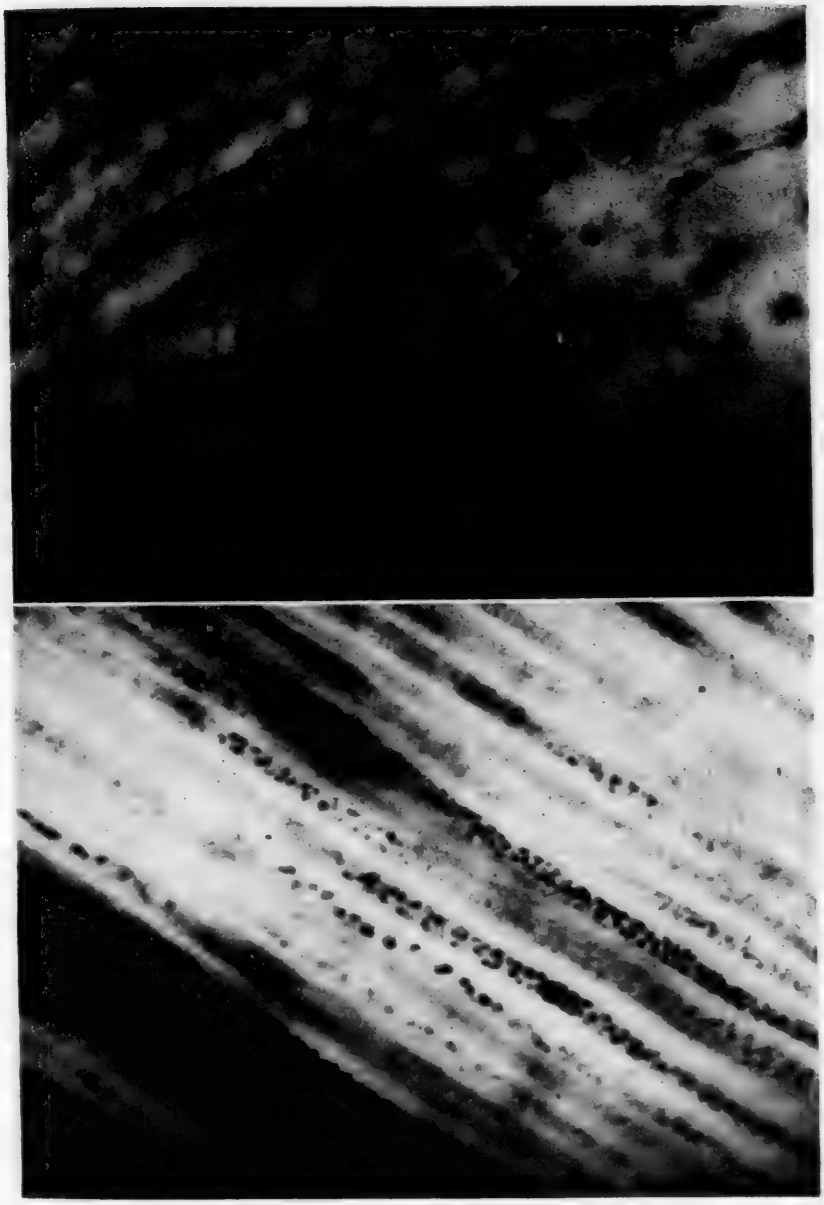

Fig. 10. Medium sized argyrol particles in dentinal fibers in the efferent zone. (4500×).
Fig. 11. Argyrol stained dentin in afferent zone with medium sized particles limited to dentinal fibers. (2025×)

CONCLUSIONS

(1) Our observations are based to date on 224 vitally stained teeth of man, dog, monkey, sheep, calf and cat.

(2) The evidence of the flow of dental lymph was not based on silver nitrate alone, but also on water soluble eosin, congo red, borax carmine, India ink, methylene blue, argyrol and potassium permanganate.

(3) Dyes penetrate metamorphosed dentin to a variable degree, but much less profusely than through young dentin.

(4) "Secondary vital staining'. of dentin and enamel, resulting from dye redistribution by the pulp proves that the dental lymph (tissue fluid) originates from this organ.

(5) Dye particles such as argyrol and potassium permanganate are transported efferently and afferently in uninjured dentinal tubules.

(6) The presence of the dental lymph diffusing in 2 directions may explain one phase of the mechanism of dentin metabolism.

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Addendum: The Editor sent this paper to Drs. Zander and Smith so as to give them an opportunity of expressing their opinion on the added evidence presented by the authors. Dr. Zander stated that he has another paper in preparation and gave his permission to quote his views as follows: "Dentin consists of a matrix and tubules. The tubules contain the cytoplasmic processes of the odontoblasts. There can be no doubt that these processes are part of living cells and have a definite task assigned to them by nature. Metabolism always takes place in living cells. There is no question therfore that dentin is a tissue in which metabolic processes occur. It must be emphazized however that the existance of dental

lymph is not a criterion for the presence or absence of metabolic processes in dentin. It is conceivable that osmosis and differences of tonicity of tissue fluids are capable of providing the mechanisms for tissue metabolism. There is evidence in other organs of metabolism. There is evidence in other organs of matabolism without two directional flow through lymph channels. The movement of fluid through the glomeruli in the kidney can be explained on purely physical and chemical grounds. The movement of fluid from the tubules of the kidney to the blood occurs not though lymph channels but through osmosis and even sometimes by methods which are not explainable because they are contrary to the laws of osmosis.

"It is true that death is often accompanied by an increase in permeability and therefore the permeability of non-vital dentin to silver nitrate solutions *can not* be used as an argument *against* the existence of lymph channels in dentin. However, it is also true that if silver nitrate penetrates dentin of non-vital teeth and metamorphosed dentin, that such penetration can be explained by a physical-chemical process and not by the two directional flow of a lymph stream."

It is evident that the difference of opinion on this subject is mainly a matter of definitions. Dr. Zander seems to believe in metabolism in dentin just as do the undersigned.

CHARLES F. BODECKER, WILLIAM LEFKOWITZ.

A DEMONSTRABLE LINE ON EXTRACTED TEETH INDICATING THE LOCATION OF THE OUTER BORDER OF THE EPITHELIAL ATTACHMENT[1]

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The line to which attention is here directed may be seen on almost any extracted tooth specimen, when prepared and examined by appropriate methods. It consists of a narrow zone along which the border of the cuticle, which is laid down by the epithelial attachment, is disintegrating and stains heavier than the normal cuticle. Although this line must have been seen previously by others, I have not found any published mention or reference indicating that it has been recognized.

The epithelial attachment was first recognized and described by Gottlieb (1) in 1921. Since that time it has come to be generally accepted in scientific dental literature and descriptions are to be found in most of the later textbooks embracing the histology and pathology of the periodontal tissues (2, 3, 4, 5, 6).

When the eruption of a tooth is complete and it has reached the normal occlusal level, the epithelial attachment consists of a band of epithelial tissue, from one to several cells thick, attached to the enamel of the rootward third or half of the anatomical crown. It is derived from, and consists of, a remaining portion of the enamel organ. At this time it extends from the gingival crevice to the cemento-enamel junction.

As the tip of the erupting tooth pushes its way through the outer enamel epithelium and the oral epithelium, these epithelial structures grow together (1, 3, 6). Thereafter the epithelial attachment, as long as it remains entirely on the enamel, consists of this band of reduced enamel epithelium, to the outer surface of which the oral epithelium is organically attached.

As the location of the epithelial attachment moves towards the apex, its width becomes narrower and narrower. Whereas, when it is first established entirely on the enamel its width is equal to from one third to one half the length of the anatomical crown, when it is far down on the cementum it is only a fraction as wide. However, at any stage of progress of the usual pathological exfoliation (periodontoclasia) there is always a band, wider or narrower, of epithelial tissue (epithelial attachment) surrounding the tooth. The outer border of this epithelial attachment marks exactly the extent to which the soft tissue attached to the tooth has receded at any place. The demonstrable line to which attention is

[1] Studies promoted by facilities to which the author has had access at the School of Medicine, Tulane University of Louisiana, and by aid for equipment and supplies provided by the University. Received for publication May 31, 1946, revised by author July 18, 1946.

called, is always located immediately adjacent to the outer border of the epithelial attachment. Therefore it accurately indicates the location of that important tissue and may be found useful in studying extracted teeth relative to periodontoclasia.

MATERIAL

Specimens may be examined soon after they are extracted; however, it is usually more convenient and more satisfactory to collect teeth, preserve them in formalin (2.5% sol.) and to examine them as desired later. If the formalin solution is changed occasionally, specimens may be kept for long periods of time and will be entirely satisfactory for study when needed. I have kept teeth preserved in this way for more than 3 years and they are still satisfactory for study in relation to both caries and periodontoclasia.

Quantities of teeth from extraction clinics or private practice may be collected and preserved in this way, for one's own studies, and for instruction and demonstration to students and others. It should be mentioned that specimens that have been stained and examined, and found interesting and instructive, can be preserved in the formalin solution for demonstration later to others.

EQUIPMENT

For satisfactory microscopic study of extracted teeth for any purpose one requires a good dissecting microscope and some facility for illuminating the object from above. I use a Zeiss stereoscopic dissecting microscope (XV) with inclined eyepieces and built-in illumination for incident light. Other means of lighting the object from above can be devised, a small spotlight being better than wide field lighting. The Nicholas illuminator (No. 31-33-49-01, Bausch & Lomb) should be almost as satisfactory for this purpose as the built-in light.

For routine work a magnification of about ×12 is preferable. I use routinely 2× objectives and 6× oculars. Although several other and higher magnifications are available, I find that I use them only occasionally and then for some special purpose.

Specimens are kept wet while they are being examined under the microscope. For this purpose a shallow glass dish, with a little water in it, is placed on the stage of the microscope. The top section of a Petri dish, outside measurements 10 cm. x 0.5 cm., is just right for this purpose.

The best instrument I have used for holding and manipulating teeth under the microscope is Rochester Pean forceps No. 522 1/4. These can be obtained from dealers in surgical instruments. A longitudinal groove cut in the grasping end of the blades greatly improves this instrument for holding the root end and manipulating tooth specimens.[2]

For handling specimens, transferring to and from staining solutions, etc., a pair of ordinary surgeon's thumb forceps, 7½″ size, is needed.

[2] (Forceps with appropriate grooves cut in them can be obtained through the surgical instrument dealers who are distributors for J. Sklar Mfg. Co., 38-04 Woodside Ave., Long Island City 4, N. Y.).

For teasing out, removing and manipulating very small particles, fine teasing needles and micro-instruments are required. These are made from ordinary fine gauge (No. 10) silver steel sewing needles which are driven (eye-end) into 4″ lengths of $\frac{3}{16}$″ wooden doweling, and then shaped and sharpened on a fine grain stone (Arkansas pocket stone No. AP 12). In manipulating small particles of cuticle and other material, such as is contemplated in this paper, I find that I use mostly a needle whetted to a spade or chisel shape and about 0.35 mm wide. Round long tapered needles with thin sharp points are also needed.

Some such instrument as the S. S. White explorer, single ended, No. 7, is sometimes useful in microscopic study of the area and tissues under consideration here.

PREPARING SPECIMENS FOR MICROSCOPIC EXAMINATION

Many different stains and staining methods may be used for preparing extracted teeth for microscopic study; some better for one purpose and others better for other purposes. Workers may employ stains with which they are accustomed to working, but for the demonstration of this line crystal violet will be found most satisfactory.

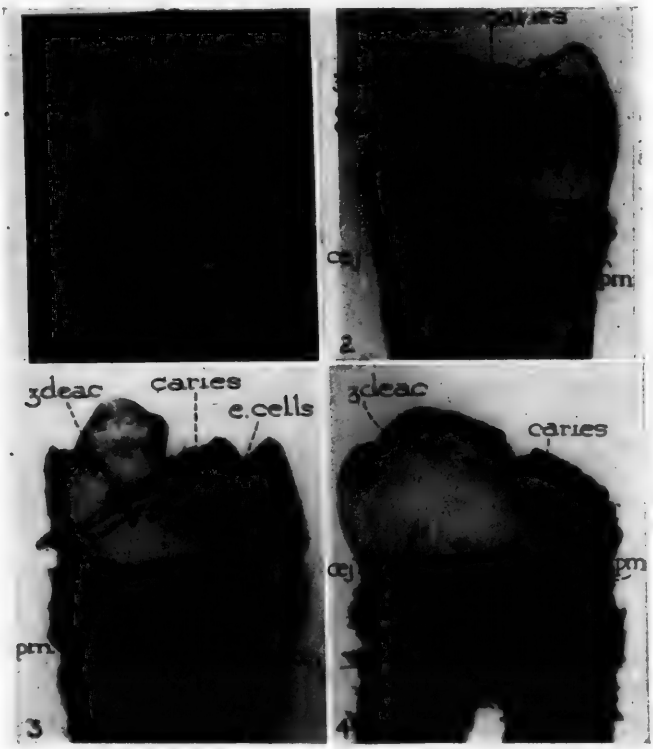
Our first objective is to see the line under consideration and learn to recognize it on different teeth. For this purpose choose several teeth, preferably specimens on which the outer border of the epithelial attachment had not moved below the cemento-enamel junction and therefore was still located on the enamel. These should be cleaned off by brushing them gently and washing them in running water. An ordinary toothbrush will serve the purpose but it is better to use a very soft brush, such as a child's size brush with nylon bristling about 0.18 mm in diameter, instead of the heavier adult size with bristles twice as thick. Very hard stiff bristles are objectionable. A little soap on the brush helps to clean off the tooth. This brushing removes most of the soft bacterial film, debris and other loose material which would be somewhat in the way of our present purpose of showing up the line most clearly. Where there are large pieces of gingiva and other soft tissue attached to the tooth, these should be trimmed away (Bard-Parker knife, No. 11 blade). This can be done better with the specimen held under the dissecting microscope.

Next place the specimens in the crystal violet solution (0.5% in water) in a small, low form, widemouth bottle or other container. Allow to stain from 1 to 5 minutes, remove, brush again, wash in running water and examine. Keep the specimen (held by the root with Pean forceps) wet while examining it, by turning it over from time to time in the water in the dish.

RECOGNIZING THE LINE

A purple stained line on the tooth will be found readily (*figs. 1–7*), its location and course varying according to the rootward progress of the outer border of the epithelial attachment at any particular place. On some specimens the line can be found only in separate places, on others it may be traced all the way around the tooth. At first, instead of expecting to recognize the line all the way around a given specimen, it is better to examine several different specimens until one

becomes familiar with the line and learns not to confuse it with other things on the tooth.



Abbreviations used:

zdeac—zone of disintegrating epithelial-attachment cuticle designating the demonstrable line which is the subject of this paper;

eac—epithelial-attachment cuticle below the disintegrating zone;

ec—enamel cuticle;

cej—cemento-enamel junction;

pm—fibers of the torn periodontal membrane remaining attached to the cementum of the extracted tooth; in this paper we are interested only in the outer borderof this membrane in relation to the zdeac;

ea—epithelial-attachment.

Fig. 1. Third molar; zdeac on enamel; note higher location on distal than mesial side; pm about at cej.

Fig. 2. Approximal side, second molar; zdeac on enamel; note how it dips below contact point where caries has already occurred; some of ea cells on eac; pm at cej.

Fig. 3. Badly decayed crown but pm still at cej; zdeac on enamel and in places near to caries.

Fig. 4. Incompletely erupted 3rd molar; caries of exposed part of crown; zdeac on enamel passes over unerupted cusp.

Next, one should prepare and examine in the same way, a number of specimens in which the soft tissues have receded, in some places at least, well below the

cemento-enamel junction. Include some in which more than half of the root has been exposed. The purple stained line on such specimens will be found even more easily and satisfactorily (*figs. 8–17*). Its course varies greatly. At one place it runs crosswise of the root, at others diagonally, or even up and down (*figs. 15–17*). In most places its course conforms to the course of the nearby border of the hard scale and calculus found on such specimens (*figs. 5, 9, 10, 11, 13, 14, 15, 16, 17, 18, 21, 22, 24, 25*).

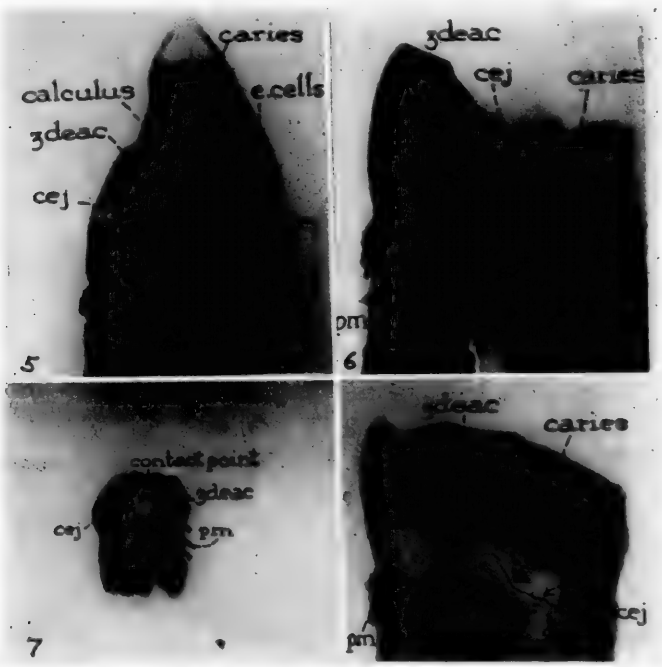


Fig. 5. Zdeac on enamel dips below calculus just below cavity; pm has receded below cej.

Fig. 6. Heavy zdeac on enamel on short, badly decayed molar; pm has receded to below (faintly shown) cej at most places.

Fig. 7. Zdeac on enamel of deciduous tooth just below worn approximal contact point.

Fig. 8. Zdeac on enamel at left, extending across and below cej, right.

It is impressive to see this line standing out so clearly on specimens on which the soft tissues had receded until only a small portion was still attached. It is present about the apex, even up to the last stages of exfoliation (*fig. 17*).

RELATION OF EPITHELIAL ATTACHMENT TO THE LINE

Having learned to recognize this line wherever it is present, one is now ready to proceed to study its relation to the epithelial attachment and to learn more of the nature of the line itself. Different procedures are available and selection will have to be determined by the circumstances and by the equipment and technical skill at the worker's command.

When a tooth is extracted, usually a few, if not many, of the cells of the epithelial attachment are torn from the soft tissue and remain attached to the tooth. Some of those at and near the outer border especially remain on the tooth. Usually most of the cells are removed by the brushing which was suggested above and intended to help bring out the line clearly. Therefore it is desirable now to

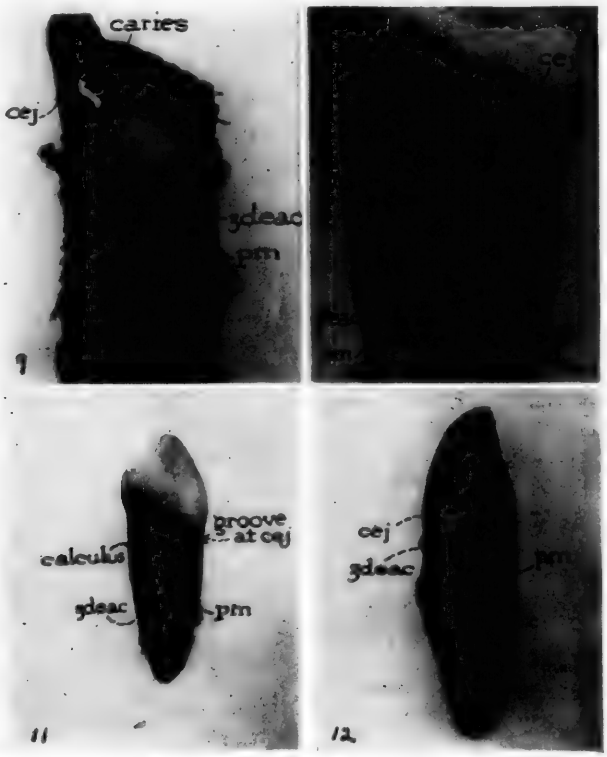


Fig. 9. Zdeac on cementum of root from which most of crown has been lost from decay line takes course around heavy lump of calculus which was broken off in extracting; note fairly uniform width of eac between zdeac and pm.

Fig. 10. Zdeac far down on cementum; course conforms to lower border of scales of calculus which remained after brushing.

Fig. 11. Zdeac far down on cementum dips and conforms somewhat to calculus above.

Fig. 12. Zdeac on cementum; cej can be seen at one place left; part of calculus was broken off; note higher point of pm corresponding to course of zdeac.

prepare selected specimens in the same way except omitting the brushing. Work first with specimens on which the outer border of the epithelial attachment is located on the enamel.

Notwithstanding the presence of more or less overhanging loose debris and bacterial film, one who already has become familiar with the deeper staining line will have no difficulty in recognizing it. At favorable locations one may find

areas or patches of raised, heavier stained material on the tooth just below the line, and between it and the adhering portions of the periodontal membrane (*figs. 2, 3, 5*). This proves later to be epithelial cells attached to the tooth. After a little experience one is able to recognize these attached cells, even under the low magnification of the dissecting microscope.

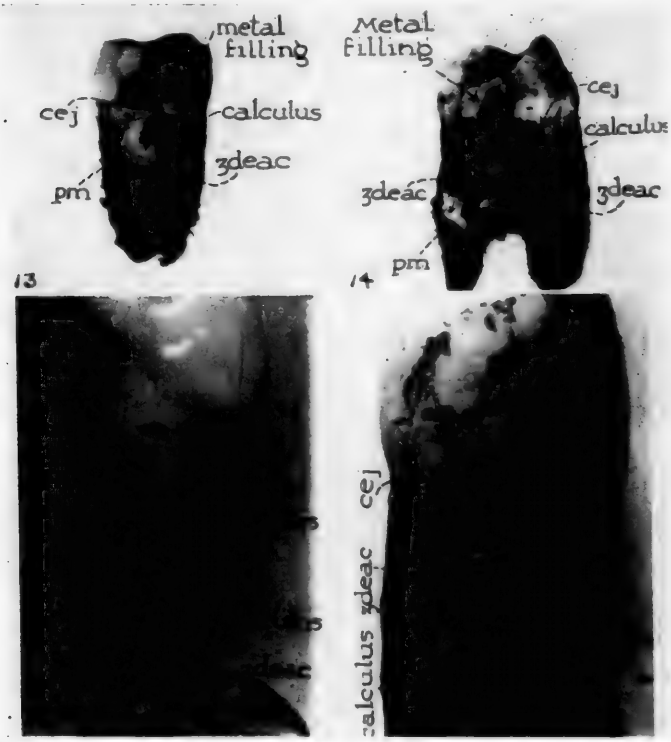


Fig. 13. Zdeac far down on cementum conforms, in general, to course of nearby border of calculus; cej shown sharply.

Fig. 14. Zdeac running crosswise or up and down around scales of calculus extending more than ¼ length of root.

Fig. 15. Zdeac running crosswise or up and down around scales of calculus extending more than ¼ length of root.

Fig. 16. Zdeac located just below cej at right, extends downward across, then curves back and down towards the apex; some of pm has been lost in preparation; calculus and plaque material on left correspond closely to course of zdeac.

For the purpose of seeing and differentiating these cells on the tooth one requires some facility for illuminating the object from above while examining it with suitable magnification under the regular microscope. I use a Bausch & Lomb surface illuminator (31-33-05-02) which can be used with objectives up to 16 mm. However, one can improvise satisfactory surface lighting for use with objectives which have working distances of 15 mm. or more, simply by directing

the light onto the object from some kind of lamps properly placed, one on either side of the microscope. A 6× objective (working distance 15.5) can be used in this way and with appropriate eyepieces gives sufficient magnification for recognition and differentiation of epithelial cells on the surface of teeth. For such

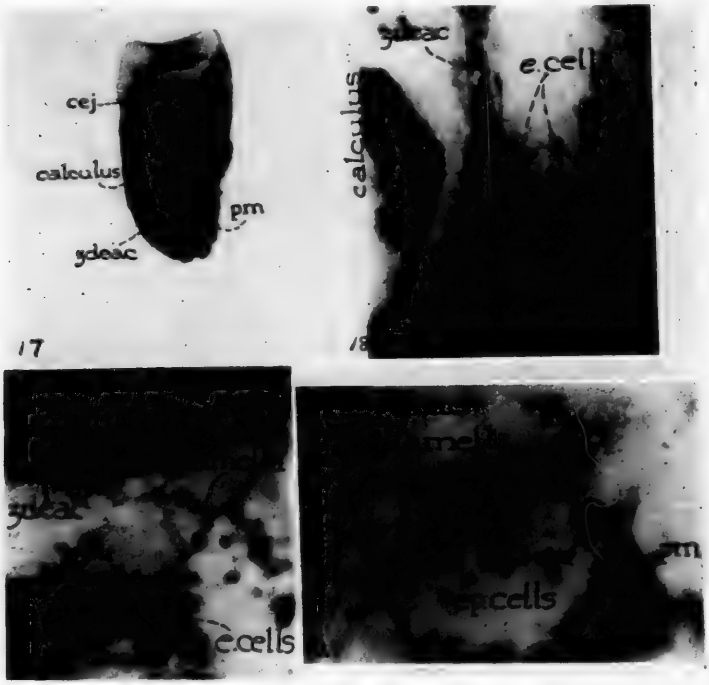


Fig. 17. Zdeac running diagonally across near apex; tooth held in by only small amount of epithelial attachment and pm as final exfoliation approaches.

Fig. 18. Area on enamel crown taken with surface illuminator, original mag. ×27; zdeac with calculus just above and some epithelial cells still attached to the eac below; approximate measurements—edge of calculus to zdeac 10 microns, zdeac 20 microns wide, zdeac to pm 50 microns.

Fig. 19. Area on enamel crown taken with surface illuminator, original mag. ×72; zdeac above, some attached epithelial cells and an enamel lamella (or crack) below.

Fig. 20. Area on enamel crown taken with surface illuminator, original mag. ×72; edge of cavity, upper left corner; zdeac faintly below to left; epethelial cells attached to eac running right up to lower side of zdeac; one heavily stained lamella (or crack).

examination the specimen is placed in a Petri dish containing just enough water to cover the tooth.

The crystal violet stain does not give as sharp differentiation of nucleus and cytoplasm as many other stains, especially such strong nuclear stains as hematoxylin. Although crystal violet will be found satisfactory for most purposes, it is suggested that those who wish other effects try 1) hematoxylin and eosin, 2) dilute Giemsa, or 3) fuchsin and toluidine blue (0.05% basic fuchsin in N/500

HCl, 3 minutes, wash, toluidine blue 1% in 20 per cent alcohol ½ to 1 minute, wash).

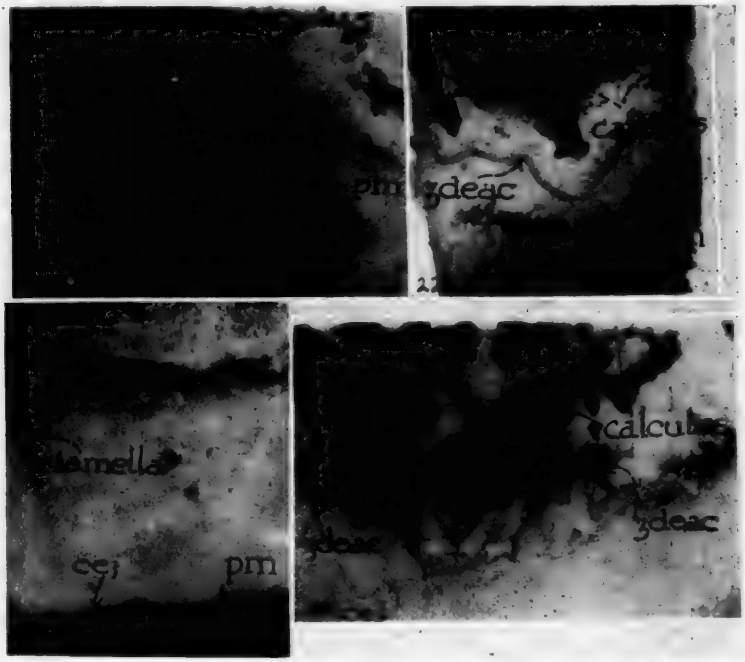


Fig. 21. Area on cementum, taken with 2 reflector lamps, original mag. ×18; zdeac curves around calculus scale; fairly uniform distance from zdeac to pm.

Fig. 22. Area far down on cementum, original mag. ×4; zdeac runs irregularly across root, conforming somewhat to nearby border of calculus scales; pm partly torn away at left.

Fig. 23. Area on crown of deciduous tooth (see *fig. 7*) taken with surface illuminator, original mag. ×27; sharp line, zdeac, left after brushing which removed outer border of the zone and some plaque material, leaving almost clear space between; one lamella (or crack) and eac extend far down to pm located at cej.

Fig. 24. Area on crown just above cej, taken with surface illuminator, original mag. ×27; zdeac has scales of calculus projecting into it; indicates how calculus crowds down eac in some places.

RELATION OF EPITHELIAL CELLS TO DENSER STAINED LINE

Examining specimens in this way the relation of the outer border of the epithelial attachment to the denser stained line, as indicated by the attached cells, can be recognized. The cells are flattened and spread out towards the lower side of the purple line (*fig. 18*). Sometimes the layer of cells at the border is only 1 or 2 cells thick. It may be several cells thick farther down. These flat epithelial cells appear to spread out to cover the surface and to be connected by projections from their reticulated cytoplasm (*figs. 19, 20*). Often mitosis can be seen, showing nuclei in different stages of division and probably indicating the active repro-

duction that must be required at this place where there is considerable loss of cells going on continuously.

EXAMINATION OF PIECES OF CUTICLE REMOVED FROM TOOTH

The relation of the outer border of the epithelial attachment to the line, as indicated by the adhering cells, and further information about the nature of the line itself can be studied better by an entirely different procedure, not requiring surface illumination for the regular microscope, but requiring somewhat more delicate technic. It consists of removing a suitable, selected piece of cuticle, including both adhering epithelial cells and the line, and restaining. When properly stained and mounted, such a preparation can be examined satisfactorily under the higher powers of the microscope, including oil-immersion objectives. Not only can the attached cells be seen and differentiated but the nature and composition of the line can be seen clearly.

Since the technic here required includes steps similar to some that are necessary in other studies of extracted teeth, it will be given in some detail. This is done, however, with the full realization that others may prefer to develop and employ methods which seem more appropriate and are more convenient for them. Select a stained specimen on which the line and some epithelial cells are located on the lower half of the anatomical crown. Under the dissecting microscope, with the point of a sharp knife (Bard-Parker, No. 11 blade) make cuts through the cuticle outlining the area selected to be removed. Include in this window or piece of cuticle to be removed, some of the attached cells and the full width of the stained line. It is better to go just a little beyond it; however, for present purposes, the much thicker bacterial material above the line should be avoided.

In some places, at least, the lines that have been cut through the cuticle can be seen under the dissecting microscope. One can select and cut in the same way, outlines of other areas which it is desired to remove and examine. Small pieces are much easier to manipulate and mount. Pieces less than 1 mm wide and 2 or 3 mm long should be used at first. Later one may wish to mount larger pieces but the manipulations become more difficult and the results are less satisfactory. Having cut around, with the sharp pointed blade, one or more areas for removal, the cuticle is loosened by immersing the tooth in hydrochloric acid solution. (For all routine work I use HCl 10 cc., formalin 5 cc., water 85 cc. Other weaker solutions of HCl are quite satisfactory. One per cent HCl can be used but its action is slower and weaker). The decalcification is carried only far enough to release the cuticle and preferably not far enough to dissolve the entire thickness of enamel, in which case the floating cuticle is more difficult to recover and handle for the present purpose. The cuticle is released more quickly on some teeth than others, but usually 2 to 5 minutes in the acid is long enough.

After the proper time in the acid, the tooth is removed with forceps, grasped by the root with the modified Rochester Pean forceps and transferred (without washing) to the dish of water on the stage of the dissecting microscope. The specimen must be handled gently and carefully, at this stage, to avoid dislodging and losing the piece of material wanted. With a teasing needle the piece de-

sired is teased loose from any connection (if any) to the surrounding membrane and dropped into the shallow water in the dish. From there it can be transferred with the flattened (0.35 mm) needle, used as a section lifter, to a drop of water on slide (or into another dish of water if several pieces are to be used) to wash out the acid. A minute or two is all that is necessary, especially if the specimen is transferred to, and washed in, a second separate drop of water on the slide.

The specimen which has lost most of the first stain in the acid is now ready for restaining. Any one of many different stains and staining methods may be employed. Fuchsin and especially carbolfuchsin is a strong stain for this cuticle. One should first look at specimens stained with it alone. It stains, not only the cuticle upon which the epithelial cells rest but even better the disintegrating border of this cuticle which constitutes our line. The much thinner layer of cuticle just above the line is also stained, more or less. Epithelial cells, both nucleus and cytoplasm, are stained intensively but can be recognized without difficulty. Our main objective here is merely to recognize and identify the cells from the epithelial attachment which remained attached to the cuticle and to observe the relation of the outer border of these cells to our line.

A convenient procedure is to put a drop of 50% glycerin (glycerin 50 cc., water 50 cc.) on a slide near one end. Add to it a small drop of carbolfuchsin. Mix with a needle and transfer the specimen into this diluted stain. Staining proceeds rapidly. One or 2 minutes is sufficient for most purposes. However, staining may be continued much longer and thereby stain more deeply, the almost transparent normal cuticle. Place a drop of 50% glycerin on the slide (or on a separate one if desired) and near it a smaller droplet in which the specimen is to be finally mounted for examination. Transfer the specimen to the large drop of glycerin and wash it, moving it about with the needle to hasten the process. Then transfer the specimen to the smaller droplet of glycerin. Spread it out and orient it by the use of teasing needles, one in each hand. Put on a cover glass.

There are advantages here in using smaller than the usual size of cover glasses. It is convenient to cut the regular $\frac{7}{8}''$ square size into 4 square pieces, with the aid of a glass marking pencil or scriber. A pair of medium point, curved forceps (Fischer No. 8-882) is useful in placing the small cover glass on the specimen. The cover glass is pressed down and any excess of glycerin is blotted away with the corner or edge of a towel.

Better effects for some purposes may be obtained by adding a small drop of crystal violet to the diluted carbolfuchsin on the slide. The disintegration of the cuticle and its separate layers may be seen sometimes better with this double stain. Any one of many other stains may be used including hematoxylin and eosin, Giemsa or the fuchsin and toluidin blue mentioned above.

COMPOSITION OF LINE

The line is seen to consist of a heavier stained zone or border of the same membrane on which the epithelial cells are attached. It consists of 2 or more layers which are broken into irregular patches, especially at the ragged outer border. At the inner border the heavier stained membrane merges into and is continuous

with the normal cuticle to which the epithelial cells are attached. Places may be found where irregularly shaped gaps or spaces seem to be broken out of one layer, leaving other layers showing more clearly (*figs. 26, 27, 28*).

In addition to the larger breaks and cracks seen in the outer part of this membrane, upon high magnification and appropriate adjustments of the light one sees

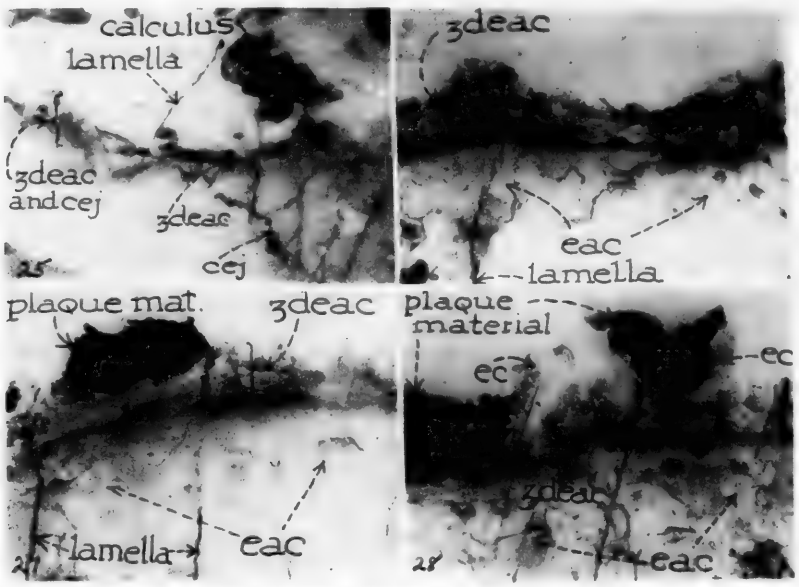


Fig. 25. Area takes in cej running diagonally across, taken with surface illuminator original mag. ×27; zdeac runs along cej at left; along middle it is stained heavily and located well above cej; scale of calculus just above; several enamel lamellae (or cracks) in part covered with eac, one in enamel from which eac has been destroyed.

Fig. 26. Piece of cuticle released by acid and teased from surface of enamel, original mag. ×72; zdeac very ragged and darker stained than eac below; at least 2 or 3 layers of the latter can be made out but still more layers of the disintegrating zone can be seen.

Fig. 27. Piece of cuticle from enamel, original mag. ×72; zdeac appears to have several layers; at least 2 layers of eac below; piece of plaque material upon ec which is continuous with and underlies eac.

Fig. 28. Piece of cuticle from enamel, original mag. ×72; zdeac and eac below from which patches of one or more layers are torn out; piece of plaque material upon ec and another piece at left folded down over the zdeac.

that the material is checkered and breaking up into still smaller divisions. Perhaps it can be spoken of as "crinkled" (*figs. 31, 31*).

Pieces loosened on one side and attached on the other may be seen. It is very evident that the cuticular material here is breaking up or disintegrating, and as a result stains heavier than the other part of the cuticle (*fig. 29*). The disintegration accounts for the heavier staining. Our line therefore consists of a portion or zone of the cuticle which is in different stages of disintegration.

On well made preparations we find that one layer, usually extremely thin, extends (occlusalward) beyond the line or zone and is actually continuous with the thin enamel cuticle which covers the entire crown ("primary enamel cuticle") (7, 8). Apparently the cuticle which disintegrates is superimposed upon, and attached to, this deeper layer which rests upon the enamel. On a favorable specimen and by delicate technic I have been able to make out at least 5 different layers or lamina of cuticle at the disintegrating zone, and I am inclined to believe a still larger number may be present in some specimens.

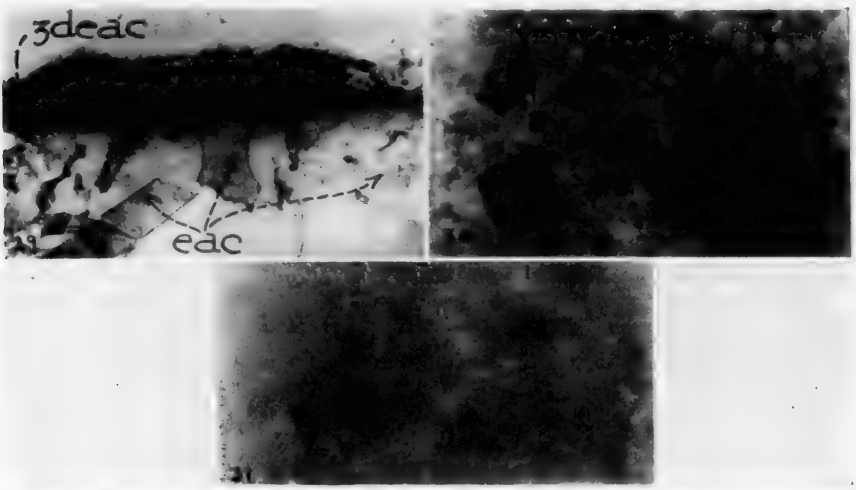


Fig. 29. Piece of cuticle from enamel, original mag. ×72; breaking up and multiple layers at this area emphasized by small condenser diaphragm opening and greater depth of focus; eac below is seen to consist of at least 2 or 3 layers; lower, left of center, a piece of this extremely thin cuticle is folded sharply back and to the side.

Fig. 30. Cuticle from enamel, field near rootward edge of zdeac, original mag. ×810; early stage of disintegration, mottled or crinkled appearance before any breaking apart has occurred.

Fig. 31. Cuticle from enamel, field a little nearer edge of zdeac than *fig. 30*, original mag. ×810; very fine mottling or crinkling, early stage of disintegration at this area.

Still further light is thrown upon this cuticle by scratching and tearing it before removing it for higher magnification. With the teasing needle one can make scratches across the heavier stained zone and actually scrape back part or all of the layers of the disintegrating cuticle. Sometimes one can scrape back (or off) all except the deepest, extremely thin layer which rests upon the enamel and is continuous with the cuticular membrane which covers all of the enamel. A specimen obtained by cutting out a window, as described above, including the area which was scratched, if successfully prepared, will show this deeper layer to be continuous, whereas the outer heavier stained layers have been scraped off from it.

ATTACHMENT CELLS AND LINE ON CEMENTUM

As the epithelial attachment moves towards the apex it crosses over the cemento-enamel junction and at a time rests partly upon enamel and partly upon the cementum below the junction line (*figs. 8, 25*). As it moves on down farther the outer border passes the cemento-enamel junction and the attachment then rests entirely upon cementum.

Gottlieb pointed out that a cuticle-like membrane underlies the epithelial attachment and, in fact, is the means by which the cells of the attachment are attached to the cementum. This cuticle is laid down or produced by the epithelial attachment and was called by him cuticula dentis, or dental cuticle, as distinguished from the (so called) primary (and the secondary enamel) cuticle.

This dental cuticle on the cementum beneath the epithelial attachment can be seen in sections which include the root and soft tissues. It is now generally recognized and accepted (**2**, 4, 5, 6).

Often there are some cells from the epithelial attachment attached to the cuticle upon the cementum. We can find some of these extending near to the lower side of the heavier stained zone of disintegrating cuticle, which is our line, just as we did when the line was located on the enamel.

The disintegrating cuticle producing the line and the normal cuticle with or without attached epithelial cells can be scraped off in the same way as when they are located on the enamel but there is no underlying primary enamel cuticle. Neither can we release the cuticle with acid as when it is located on the enamel. The acid does not cause complete loss of cementum, as it does enamel, and thereby free the cuticle. Therefore the cuticle remains firmly attached to the cementum, although the latter may be completely decalcified.

The epithelial cells can be differentiated and the nature of the line on the cementum can be observed directly upon the tooth by suitable magnification under the regular microscope, as described above for specimens wherein they are located on the enamel. It is also possible to tease off very small particles of this membrane with cells attached to it, or the line itself, and to stain, mount, and examine them under higher magnifications. Although such preparations are less satisfactory than those from enamel surfaces, it is quite possible to recognize some of the epithelial cells and to see that the line consists of the same kind of heavier stained, disintegrating, laminated, cuticular membrane.

NAME SUGGESTED FOR CUTICLE AND THIS LINE

This cuticle, the disintegrating border of which produces the line to which we dierect attention, underlies the epithelial attachment, is laid down or produced by the epithelial attachment and attaches the cells of this tissue to the tooth, either on the enamel or on the cementum. Therefore I propose to call it the epithelial-attachment cuticle; and this demonstrable line, the zone of disintegrating epithelial-attachment cuticle or zdeac.

Those who do not accept this term may prefer to call this line the zone of disintegrating secondary cuticle or the zone of disintegrating dental cuticle, in conformity with names applied to this cuticle by Gottlieb. Or, if one adopts the

name for the cuticle suggested by Glickman and Bibby (9) he may prefer to call it the zone of disintegrating transposed-crevicular cuticle. Whatever name is accepted it should be clearly understood that the demonstrable line to which attention is here directed, consists of a narrow zone (or band) along which the cuticular membrane which is always present beneath the epithelial attachment, whatever the location of the latter on the tooth, is breaking down or disintegrating; and further that this cuticle, when located on the enamel overlies, is attached to, but is different from, the so-called primary enamel cuticle (*fig. 23*).

It should also be understood that whether there are many, few or no cells from the epithelial attachment found on a given specimen, the deeper stained line exactly conforms to, and indicates the location of, the outer border of the epithelial attachment before the tooth was extracted. After one has learned from sufficient experience that the cells of the attachment come right up to, but do not overlap this line, it serves as a useful landmark indicating exactly the extent of recession of this tissue at any particular place.

SUMMARY

1. Attention has been directed to a demonstrable line on extracted teeth which is a good guide to the location of the outer border of the epithelial attachment. Technic for demonstrating it was given.

2. The line consists of a denser stained border or zone of the cuticular structure upon which the epithelial attachment rests. The denser staining is due to disintegration of this part of the cuticle.

3. It is proposed to call the cuticle upon which the epithelial attachment rests and which is produced by the epithelial attachment, the epithelial-attachment cuticle and this line, the zone of disintegrating epithelial-attachment cuticle or zdeac.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
SCIENTIFIC PROCEEDINGS OF THE NEW YORK SECTION[1]

FACULTY CLUB, COLUMBIA UNIVERSITY, JUNE 5, 1946[2]

SAMUEL C. MILLER, *Editor, New York Section*

1. THE ILLUSORY SHEATH OF NEUMANN IN DENTIN. *Charles F. Bodecker, School of Dental and Oral Surgery, Columbia University, New York.* Published in *J. D. Res.* **25:** 176, 1946 (abstract).

2. EXPERIMENTAL AMELOTOMY. *William Lefkowitz, Harry Shapiro, and Charles F. Bodecker, School of Dental and Oral Surgery, Columbia University, New York.* Published in *J. D. Res.* **25:** 182, 1946 (abstract).

3. BILATERAL FIBRO-OSTEOMA OF THE MAXILLAE IN A MONKEY[3]. *Robin M. Rankow*[4], *New York.* A report of two unusual, bilateral symmetrical osteodystrophies of the maxillae in a monkey. Roentgenographic examination of these maxillary protruberances reveals two large bilateral, hazily-diffuse, non-erosive areas, devoid of cortical margins. These growths are expanding anteriorly and inferiorly. Grossly the mucous membrane is intact and unchanged except for marked thickening. On sectioning, the contents of the bony tumor appear dirty-gray, coarse, granular and cut readily with the dental burr. Microscopic examination of the overlying mucous membrane indicates as intact, somewhat squat but essentially normal epithelium. The sub-epithelium is densely fibrous with sparse nuclei and occasional blood vessels. There are narrow, rregular bony trabeculae throughout this outer fibrous tissue. The central portions of these trabeculae are still calcified and deeply staining. They are encircled by layers of osteoid tissue of varying thickness, in a fashion similar to that seen in osteomalacia. In contrast, a specimen from further within the lesion is typical of the many small eosinophilic trabeculae of a fibro-osteoma. The marrow spaces have been replaced by dense, non-inflamed connective tissue. There is little evidence of lacunar resorption. Few osteoclasts are present. A tentative histopathological diagnosis of bilateral fibro-osteoma, with a peripheral osteomalacia, is suggested.

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[2]Presiding—Daniel E. Ziskin.

[3]Material supplied by Dr. W. Redett Hatfield and prepared in the Division of Oral Pathology, School of Dental and Oral Surgery, Columbia University, New York.

[4]Guest.

4. Measurement of the Frankfort-Mandibular Plane Angle, the Frankfort-Orbital Plane Angle and the Incisor-Mandibular Plane Angle. *J. A. Salzmann, New York.* The Frankfort-Mandibular Plane Angle has been demonstrated by Tweed to be one of the key measurements in orthodontic classification, diagnosis and treatment planning. Orthodontic diagnosis also requires the establishment of the relationship of the dental arches to the orbital plane. The foregoing must be completed by the measurement of the Incisor-Mandibular Angle as described by Margolis, in order to obtain complete dento-facial measurements in orthodontics. A method is presented for obtaining direct measurements of the above landmarks and angles on a three dimensional plane.